SPECIES SPECIFIC DNA MARKERS TO MONITOR GENE FLOW BETWEEN RED JUNGLE FOWL (*Gallus gallus*) AND DOMESTICATED CHICKEN (*Gallus gallus domesticus*)

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THESIS

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BY

VINAY KUMAR

Dr. JOSE MATHEW
Lecturer
Department of Biotechnology
Bundelkhand University
Jhansi (U.P.)

Dr. DEEPAK SHARMA
Principal Scientist
Avian Genetics & Breeding
Central Avian Research Institute
Izatnagar, Bareilly (U.P.)

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JHANSI (U.P.) INDIA
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Dedicated to...

My beloved Parents

CERTIFICATE

This is to certify that the research work in this thesis entitled "**Species specific DNA markers to monitor gene flow between red jungle fowl (*Gallus gallus*) and domesticated chicken (*Gallus gallus domesticus*)**" submitted by **Mr Vinay Kumar** for the award of the **Doctor of Philosophy in Biotechnology** of Bundelkhand University, Jhansi, Uttar Pradesh is an original work carried out by the candidate himself under our supervision and guidance. He has fulfilled all the requirements of the ordinance relating to the award of PhD degree of the University.

It is further certified that **Mr Vinay Kumar** has put in more than 200 days attendance in Department of Biotechnology, Bundelkhand University, Jhansi as required under the relevant ordinance.

Dr Jose Mathew
(Co-guide)
Lecturer
Department of Biotechnology
Bundelkhand University
Jhansi (U.P.)

Dr Deepak Sharma
(Guide)
Principal Scientist
Avian Genetics & Breeding
Central Avian Research Institute
Izatnagar, Bareilly (U.P.)

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(Vinay Kumar)

Abbreviations

%	:	Percent
µg	:	Micro gram
µl	:	Micro liter
µM	:	Micro molar
AFLP	:	Amplified Fragment Length Polymorphism
AS	:	Aseel
bp	:	Base pair
cm	:	Centimeter
cM	:	Centimorgan
DW	:	Distilled Water
DNA	:	Deoxy ribose nucleic acid
dNTP	:	Deoxy – nucleoside triphosphate
EDTA	:	Ethylene di amine tetra-acetic acid
Fig.	:	Figure
g	:	Gram
hrs	:	hours
i.e.	:	That is
Kb	:	Kilobase
M	:	Molar
MASA	:	Minisatellite Associated Sequence Amplification
mer	:	Oligomere
mg	:	Milli gram
min	:	Minute
ml	:	Milli liter
mM	:	Milli molar
ng	:	Nano gram
ng/µl	:	Nanogram/Micro liter
nm	:	nanometer
nt/nts	:	Nucleotide/Nucleotides

°C	:	Degree Centigrade
OD	:	Optical Density
pH	:	H ion concentration
PIC	:	Polymorphism Information Content
pmol	:	Pico mole
RAPD	:	Random Amplified Polymorphic DNA
RC	:	Red Cornish
RE	:	Restriction Enzyme
RFLP	:	Restriction Fragment Length Polymorphism
RJF	:	Red Jungle Fowl
RL	:	Reaction Ligation
rpm	:	Rotation per minute
s	:	Seconds
STS	:	Sequence Tagged Site
Taq	:	Thermus aquaticus
U	:	Unit
UV	:	Ultra violet
V/cm	:	Volt per Centimeter
vs.	:	versus
w/v	:	Weight/Volume
WLH	:	White Leghorn

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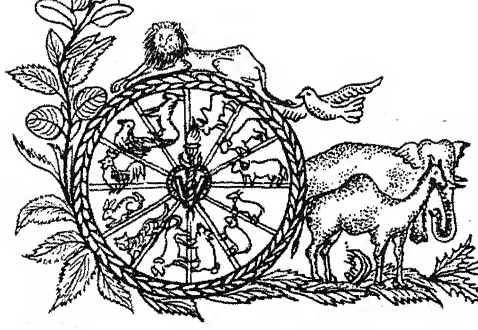
Introduction



Introduction

India and neighboring countries have been identified as one of the original homes of the RJF. It is widely accepted that all populations of domesticated chickens descend from a single ancestor, the Red Jungle Fowl (RJF) (*Gallus gallus*), originating in Southeast Asia. Though it has been claimed that other wild species of *Gallus* might have contributed to the domesticated chicken, but the more widely accepted view is that RJF (*Gallus gallus*) alone is sufficient to account for the maternal ancestry of the domesticated chicken (Hillel *et al.,* 2003; Kanginakudru *et al.,* 2008).

On the bases of phenotypic traits and geographic distribution of the populations *Gallus gallus* (RJF) consists of five sub-species: *G. gallus gallus* (Indochina RJF), *G. gallus bankiva* (Java RJF), *G. gallus spadicus* (Myanmar RJF), *G. gallus jabouillei* (Viatnam RJF) and *G. gallus murghi* (Indian RJF). Beside these five, the domestic chicken (*G. gallus domesticus*) is also considered as a sub-species of species *Gallus gallus*. The evolution of chicken includes first the evolution and speciation of wild ancestor followed by domestication of the wild species and finally diversification towards development of new breeds with an additional process of subsequent replacement of wild genes through hybridization with feral or free-ranging domestic stock (Peterson and Brisbin, 1999).

The domestic chicken (*Gallus gallus domesticus*) originated from southwestern Asia and was first introduced into India in about 3000 BC. Chickens are also depicted in Babylonian carvings of about 600 BC and are mentioned by ancient Greek writers, particularly Aristophanes in 400 BC. Since, the divergence between Red Jungle Fowl (RJF) and Domestic Fowl (DF) is presumed to have originated some 8000 years back and during all these years, its genome has undergone tremendous changes either natural or intentional especially regarding its production potential and disease resistance. Since all the existing genetic diversity in chicken seems to be originated from single source i.e. red jungle fowl, the genome of RJF may serve as gene pool for chicken biodiversity. Unlike other domestic species, where the ancestor from which the present day animals evolved are not existing, chicken offers a unique

system for identifying random as well as specific genomic differences. Such differences may serve as species-specific markers and/or might be related with some specific function such as production potential, diseases resistance etc.

Some threats have been expressed that the wild RJF populations may be genetically contaminated leading to an inference that there may not be any pure RJF populations in the wild (Peterson and Brisbin, 1999). These observations were based upon examinations of skins collected in the past from various parts of Asia and preserved in various museums in America and Europe. The authors of this paper contend that in the past, the wild RJF populations have hybridized with domestic, feral and/or domestic stock, especially near by the villages causing the introgression of domestic genes into the wild populations. The skins that were examined by Peterson and Brisbin (1999) showed lack of phenotypic traits, which characterize true wild RJF (Morejohn, 1968). However, this reports suffers from several controversial issues regarding the true representative of the wild population, especially in India and Myanmar by the samples used in the study. But, the apparent sampling inadequacy, the threat of hybridization to the RJF in India is real and needs to be addressed urgently. It calls the need of developing the species-specific markers, especially DNA markers, which can provide the dependable standards for judging the purity of red jungle fowl and to detect any contamination from feral chicken populations.

The chicken has a proud history, both in genetic research and as a source of food. The chicken was the first among farm animals to have its genome sequenced. Chickens have played an important role in some major advances. For instance, Bateson (1902) used chickens to first demonstrate Mendelian inheritance of traits in animals. Selection programs and cross-breeding experiments in chickens have played an important role for testing and further developing theories in quantitative genetics. The chicken (*Gallus gallus*) is also an important model organism and serves as the main laboratory model for about 9,600 extant avian species and a very useful model for comparative genomics because it represents the closest taxonomic out-group to mammals. Domestic chickens representing four evolutionary lineages: egg-type, game-type, meat-type and bantam. One of the important unsolved questions is which chicken breed groups are the closest to Red Jungle Fowl (*G. gallus*) and, therefore, what types of domesticated fowls are the most ancient. There are, however, a number of difficulties in answering these questions, and it has been impossible so far to

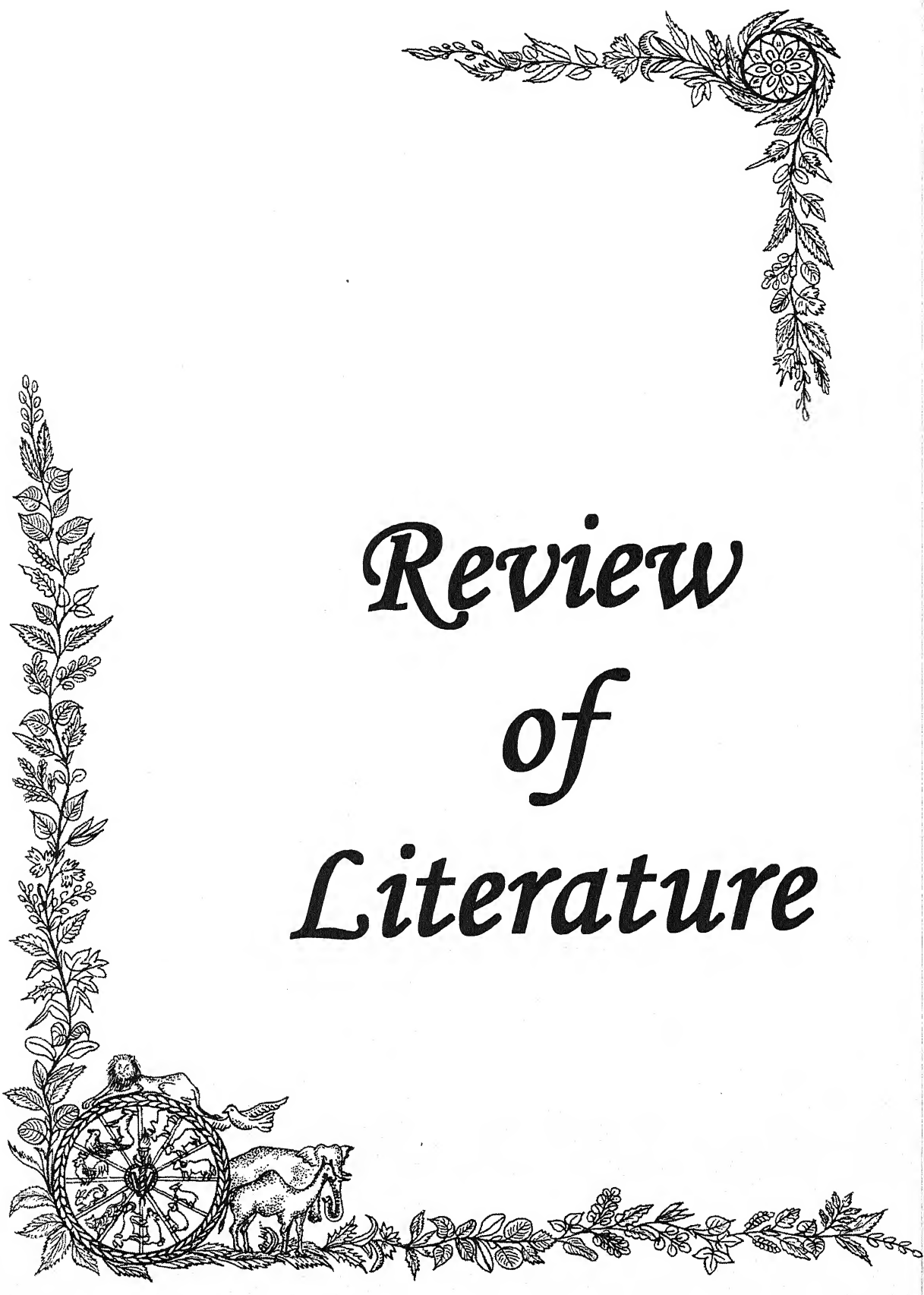
establish what evolutionary branches of chicken breeds are the closest to their major progenitor (Moiseyeva *et al.,* 2003).

The advances in biotechnology have provided number of DNA marker systems, which can detect the genetic polymorphism existing at genomic level. These markers may be the southern hybridization based using cloned sequence representing specific gene as primer (Restriction fragment length polymorphism- Botstein *et al.,* 1980), random cloned sequences representing variable number of tandem repeats either minisatellites (Jeffreys *et al.*, 1985) or short synthetic oligoes or PCR based using either random primers (Random amplified polymorphic DNA / Arbitrarily primed polymerase chain reaction- Williams *et al.*, 1990; Welsh and McClelland, 1990)) or specific primers flanking highly variable short tandem repeat (STR) region (Weber and May, 1989; Litt and Luty, 1989). A relatively new DNA marker is Amplified fragment length polymorphism (Vos *et al.*, 1995) which includes the restriction digestion as well as PCR amplification. Most of these markers have very well been used in detecting genetic polymorphism between poultry / chicken populations.

Among these markers, microsatellite markers seemed to be marker of choice not only for genome mapping but also in genetic diversity studies as it detects the polymorphism at specific region and this variation may be associated with the functional genes. Further, the AFLP markers have not been used much in such applications, but offer a great potential for such applications. The objectives of this study were to analyze phylogenetic relationships between RJF and domestic chickens using microsatellite and AFLP markers and to identify population-specific markers in RJF and domestic chicken breeds.

More specifically, **the objectives** of this study were:

1. **To detect polymorphism between red jungle fowl and domesticated chicken using microsatellite and AFLP markers.**
2. **To identify RJF specific microsatellite and AFLP markers.**
3. **To understand phylogenetic relationships between RJF and domesticated chickens.**
4. **To monitor gene flow between red jungle fowl and domesticated chickens using microsatellite and AFLP markers.**



Review of Literature

Review of Literature

2.1. Chicken genome

The chicken genome comprises 39 pairs of chromosomes, eight pairs of macrochromosomes, one pair of sex chromosomes (Z and W), and 30 pairs of microchromosomes. The size of the chicken genome is estimated to be 1.2 $X10^9$ base pairs. The linkage map of chicken contains at least 1,889 loci within 50 linkage groups, that span approximately 3,800 cM of the chicken genome (Groenen *et al.,* 2000). Therefore, 1 cM is approximately equivalent to 300 kb of DNA in the chicken. In contrast, 1 cM in humans is about 1,000 kb of DNA, and thus, the chicken genome is about one-third the size of the human genome.

2.2. DNA markers and its applications

Development of the polymerase chain reaction (PCR; Mullis *et al.,* 1986) was a technological breakthrough in genome analysis because it enabled the amplification of specific fragments from the total genomic DNA. Compared to other techniques, PCR based DNA markers are less labour- and time-consuming, and provide an estimate of genetic similarity by direct sampling from the entire genome with unprecedented precision. Most widely applied DNA marker techniques differ not only in principle, but also in the type and amount of polymorphism detected (Russell *et al.,* 1997). Techniques such as non- PCR based restriction fragment length polymorphisms (RFLPs; Botstein *et al.,* 1980) and PCR-based microsatellites or simple sequence repeat polymorphisms (SSRs; Tautz, 1989) possess the ability to distinguish multiple bands (alleles) per locus, thus giving more information on a single locus. By contrast, individual bands detected with PCR-based fingerprinting techniques, such as randomly amplified polymorphic DNA (RAPDs; Williams *et al.,* 1990) and amplified fragment length polymorphisms (AFLPs; Vos *et al.,* 1995), are scored on a biallelic basis, as marker band present or absent. The major advantage of fingerprinting techniques is that multiple marker bands – fingerprints – are generated in a single assay.

The majority of genetic markers in the chicken are molecular DNA markers. The DNA markers are of two types: genes with known functions (Type I) and

anonymous DNA segments (Type II), which include the highly repetitive microsatellites, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), CR1 retrotransposon elements, and others. Currently there are approximately 350 Type I markers present in chicken genes (Groenen *et al.,* 2000). In contrast, the Type II markers have received considerably more attention and they have been the marker of choice for genetic mapping, genetic diversity, QTL searches and also for phylogenetic relationships analysis. Generally, Type II markers are favored because they are highly polymorphic, and high throughput. PCR-based assays could be used for genotyping of individuals (Emara and kim, 2003). In the chicken, at least 801 microsatellites have been placed on the consensus map (Groenen *et al.,* 2000).

2.2.1. Microsatellite Markers and its application in detecting genetic polymorphism

The work has been conducted towards establishing the genetic relatedness among the red jungle fowl and domesticated chicken using molecular genetical approach. The use of microsatellites has become a standard technique for molecular genetic evaluation and mapping of chickens (e.g., Khatib *et al.,* 1993; Cheng, 1997). Microsatellites consist of tandem repeats of core sequences of di-, tri-, or tetra-nucleotide units. The polymorphic variants are likely generated by unequal crossover between the repeat units during meiosis. Microsatellites are used in a wide range of applications in the genetic evaluation of chicken lines, including bulk-segregate analysis (eg., Khatib *et al.,* 1994) and estimation of genetic relationships of native chicken populations (Ruyter-Spira *et al.,* 1997; Takahashi *et al.,* 1998).

Microsatellite markers are short DNA fragments of usually less than 100 bp and made up of tandem repeats of 1-6 bp fragments, thus also known as short tandem repeats (STR). Microsatellites can be analyzed by PCR amplification of a single tandem repeat locus using primers that anneal at its flanking regions. The PCR amplified fragments expressing the size polymorphism are the alleles at the given microsatellite locus (Weber and May, 1989; Litt and Luty, 1989). Major advantages of these markers are that they are randomly distributed, highly polymorphic, non-functional, so not subjected to selection and locus specific. They are abundant and are co-dominantly inherited, assayable easily by PCR method and suitable for automated analysis. In poultry, these markers are markers of choice in linkage analysis or genome mapping (Cheng and Crittenden, 1994; Groen *et al.*, 1994; Groenen *et al.*,

2000). Recently, they are also being used for identification of quantitative trait loci (QTL) and evaluation of genetic diversity within and between populations. There are several examples of genetic diversity studies in chicken employing microsatellites.

Crooijmans *et al.*, (1996) studied the microsatellite polymorphism in nine highly selected commercial broiler and six highly selected layer lines. The average number of marker alleles was 5.8 over all lines, 5.2 over broiler lines, and 3.0 over layer lines. The average number of marker alleles within a line was 2.9, 3.6 and 2.0 for all, broiler, and layer lines, respectively.

Vanhala *et al.*, (1998) used nine microsatellite markers in eight lines of different genetic origin; White Leghorns formed one group, two Finnish landraces formed a second group and the Rhodes Island Red together with broilers lines made up a third group. All microsatellite loci were polymorphic and the number of alleles ranged from 4 – 13 per locus and 1 –10 per line.

Based on eight microsatellites isolated from a chicken microsatellite DNA enriched library, 10 native Japanese breeds were clustered into three groups, which corresponded, to their known ancestry (Takahashi *et al.*, 1998). Zhou and Lamont (1999) analyzed 23 highly inbred lines derived from White Leghorns and exotic breeds using 42 microsatellite loci. Estimates of band sharing (0.74 – 0.96) were largest between jungle fowl and all other lines examined. In all cases, the results were in accordance with the known genetic relationships between these lines. Thus, they concluded that the use of microsatellites for the study of genetic biodiversity was accurate and reliable.

Ponsuksili *et al.*, (1999) measured genetic distances between 12 chicken lines comparing multilocus DNA fingerprints and multiple single locus microsatellite analysis and found that DNA fingerprints and microsatellites provided similar estimates and heterozygosity. Using 22 microsatellites, Wimmers *et al.*, (2000) studied the genetic variability of various indigenous chicken populations of India, Nigeria, Bolivia and Tanzania. They observed that all populations showed high levels of heterozygosity for microsatellite markers and a range of 2 –11 alleles per locus were detected.

Kaiser *et al.*, (2000) used two independent broiler chicken populations and genotyped with microsatellite markers to determine genetic polymorphisms within and among broiler populations. The 59 primer sets selected for this study provided wide genomic coverage. All 59 primer sets amplified a polymerase chain reaction

product in Population L, whereas 57 primer sets produced a product in Population C. The average allele number per line per microsatellite was 2.8 and 2.9 for Populations L and C, respectively. Considering the 57 primer pairs generating product in both lines, 72.3% of the total alleles were unique to one or the other population.

Romanov and Weigend (2001) studied the genetic variation and genetic distances between various populations of domestic and jungle fowl using microsatellite markers. In total, they genotyped 224 individuals of 20 populations for 14 microsatellite markers covering 11 linkage groups. Of the 14 microsatellite loci, the number of alleles ranged between 2 -21 per locus with a mean of 11.2 alleles per locus. They reported that the red jungle fowl (*Gallus gallus*) formed a separate branch and demonstrated a specific allele distribution when compared with domestic fowl breeds. The second branch comprised commercial layer lines and chicken breeds that were subject to intense selection in the past or had common ancestral breeds with commercial strain. The third group encompassed the German native breed populations.

In a project on the biodiversity of chickens funded by the European Commission (EC), Hillel *et al.,* (2003) assessed the genetic variation within and between 52 populations from a wide range of chicken type. Twenty two dinucleotide microsatellite markers were used to genotype DNA pools of 50 birds from each population. The polymorphism measures for the average, the least polymorphic population (inbred C line) and the most polymorphic population(*Gallus gallus spadiceus*) were, respectively, as follows: number of alleles per locus, per population: 3.5, 1.3, and 5.2; average gene diversity across markers : 0.47, 0.05, and 0.64; and proportion of polymorphic markers. 0.91, 0.25 and1.0. They observed that these were in good agreement with the breeding history of the population. They reported that unselected populations were more polymorphic than selected breeds such as layers. Thus DNA pools are effective in the preliminary assessment of genetic variation of Populations and markers.

Olowofeso *et al.,* (2005) used 15 microsatellite markers to measure some genetic parameters within and between four Haimen chicken populations: Rugao, Jiangchun, Wan-Nan and Cshiqishi. The mean allele number for all loci ranged between 5.73±0.85 (Cshiqishi) to 6.00±0.74 (Rugao) and 6.00±0.84 (Jiangchun) with across populations for all loci equals 5.88±0.06; while among loci heterozygosities (H) ranged from 0.6486±0.06 (Wan-Nan) to 0.7017±0.03 (Jiangchun) and across

populations, the average heterozygosity (H) was 0.6828±0.01. The mean effective allele number ranged from 3.96±0.60 (Wan-Nan) to 4.11±0.47 (Rugao); while the mean PIC have values between 0.6068±0.06 (Wan-Nan) to 0.6509±0.04 (Jiangchun).

Bo *et al.,* (2006) genotyped a total of 720 individuals of 12 indigenous chicken populations, geographically localized in Southern China using 30 microsatellite markers to evaluate the genetic variation and genetic distance between populations. All microsatellites were found to be polymorphic. A total 238 alleles were found at 30 microsatellite loci across 12 populations. The number of alleles per locus and effective number of alleles per locus ranged from 4 to 11 and 2.157 to 8.019, respectively. The average expected heterozygosity (H_E) was 0.669, while the average observed heterozygosity (H_O) was 0.764. The polymorphism information content (PIC) has values between 0.560 and 0.641. Using Nei`s standard distance, genetic distance calculated ranged between 0.088 (Guanxi Sanhuang *vs.* Nandan Yao) and 0.495 (Huiyang Beard *vs.* Zhangzhou Game). The expected heterozygosity is lower than that observed heterozygosity for all populations.

Cuc *et al.,* (2006) assessed genetic diversity of H'mong chickens, a local breed in the mountainous areas of Northern Vietnam. A subset of thirty-six chickens from the three villages was genotyped at 29 microsatellite loci. A total of 186 alleles were observed across all populations. The mean number of alleles was 6.41 per locus and ranged from 2 (MCW103 and MCW222) to 15 (LEI234). Heterozygosity varied from 62.7% to 66.8% for the three populations. The Nei's, Reynold's and Cavalli-Sforza distance measures showed Chieng-Chang to be more distant from the two geographically close populations.

Shahbazi *et al.,* (2007) characterized five native chicken populations located in the northwestern (West Azerbaijan), northern (Mazandaran), central (Isfahan, Yazd), and southern (Fars) provinces of Iran using five polymorphic microsatellite markers. The number of alleles ranged from three to six per microsatellite locus. All populations were characterized by a high degree of genetic diversity, with the lowest heterozygosity found in the Isfahan population (62%) and the greatest in the populations from West Azerbaijan and Mazandaran (79%). The largest Nei's unbiased genetic distance was found between the Isfahan and Fars populations (0.696) and the smallest between the Mazandaran and Yazd populations (0.097). The Isfahan population was found to be the most genetically distant among all populations

studied. These results serve as an initial step in the plan for genetic characterization and conservation of Iranian native chickens.

Tomar *et al.,* (2007) detected genetic polymorphism between red jungle fowl (RJF) and domestic chicken i.e. Aseel (AS), Red Cornish (RC), White Leghorn (WL) using 5 microsatellite markers i.e. ADL 237, LEI 65, LEI 113, MCW 156 and ROS 54. The number of alleles per locus amplified ranged from 3 (LEI 113) to 6 alleles (ADL 237 and LEI 65). In general, the size of alleles ranged from 96 bp to 290 bp and the majority of alleles were in the size range of 110 bp to 2756 bp. The mean within-breed genetic similarity in AS, RC, WL and RJF populations were 0.646, 0.659, 0.759 and 0.693, respectively. Between breed genetic similarity estimates pooled over different micro satellite markers ranged from 0.421 between RJF and WL to 0.492 between RJF and RC. In general, RJF showed lower genetic similarity with all the other three breeds in comparison to other combinations and among three breeds, it showed maximum genetic similarity with Aseel. Similarly, the between breed genetic distances estimates pooled over different microsatellite markers ranged from 0.256 (AS and RC) to 0.856 (RJF & WL).

Bao *et al.,* (2008) analysed genetic diversity and phylogenetic relationships among 568 individuals of two red jungle fowl subspecies (*Gallus gallus spadiceus* in China and *Gallus gallus gallus* in Thailand) and 14 Chinese domestic chicken breeds using 29 microstaellite loci. A total of 286 alleles were detected in 16 populations with 29 microsatellite markers and the average number of the alleles observed in 29 microsatellite loci was 9.86±6.36. The overall expected heterozygosity of all population was 0.6708±0.0251, and the number of population deviated from Hardy-Weinberg equilibrium per locus ranged from 0 to 7. Reynolds' distance values varied between 0.036 (Xiaoshan chicken-Luyuan chicken pair) and 0.330 (*G. gallus gallus*-Gushi chicken pair). An unrooted consensus tree was constructed using the neighbour-joining method and the Reynolds' genetic distance. Chahua chicken and Tibetan chicken had closer genetic relationship with these two subspecies of red jungle fowl than other domestic chicken breeds. *G. gallus spadiceus* showed closer phylogenetic relationship with Chinese domestic chicken breeds than *G. gallus gallus*.

Kaya and Yildiz (2008) analysed genetic diversity of the Turkish native chicken breeds Denizli and Gerze using 10 microsatellite markers. They genotyped a total of 125 individuals from five subpopulations. Among loci, the mean number of alleles was 7.5, expected heterozygosity (H_E) was 0.665, PIC value was 0.610, and

Wright's fixation index was 0.301. (H_E) was higher in the Denizli breed (0.656) than in the Gerze breed (0.475). The PIC values were 0.599 and 0.426 for Denizli and Gerze, respectively. A phylogenetic tree was constructed using genetic distance and the neighbor-joining method. They suggested that Denizli and Gerze subpopulations have a rich genetic diversity and concluded that the information about Denizli and Gerze breeds estimated by microsatellite analysis may also be useful as an initial guide in defining objectives for designing future investigations of genetic variation and developing conservation strategies.

Kanginakudru *et al.,* (2008) analysed 76 Indian birds that included 56 *G. g. murghi* (RJF), 16 *G. g. domesticus* (domestic chicken) and 4 *G. sonneratii* (Grey JF) using 11 microsatellite markers. A total of 197 alleles were detected, of which, 106 were from *G. g. murghi*, and 59 were from *G. g. domesticus* chicken. The total number of private alleles was maximum in *G. g. murghi* followed by *G. g. domesticus* and *G. sonneratii*, respectively. Populationwise mean number of alleles per locus ranged from 2.91 (*G. sonneratii*) to 6.09 (Birshi Kargah population of *G. g. murghi*, B-RJF), with a mean heterozygosity ranging from 0.481 (Kalesar - RJF) to 0.600 (Birshi Kargah - RJF). Population-wise Nei's genetic distance calculated using GenAlEx program showed a higher average distance between *G. sonneratii* and *G. g. domesticus* (2.099) than between *G. sonneratii – G. g. murghi* (0.758) and *G. g. murghi – G. g. domesticus* (1.695) combinations. Intra-population average distances were lower for both domestic and RJF groups than across the population distances, which is consistent with the observation that among population variation was more than within population variation.

Fang *et al.,* (2009) used 30 microsatellite markers in a genetic diversity study of Qingyuan partridge chicken. A total of 154 alleles were detected using these microsatellite markers. All the microsatellites were polymorphic, with mean allelic number of 5.1, ranging from 2-9 alleles per locus. The expected heterozygosity in the population ranged between 0.500 and 0.839, with mean of 0.685, indicating considerable genetic variation in this population.

2.2.2. AFLP Markers and its application in detecting genetic polymorphism

Amplified fragment length polymorphism (AFLP), a multilocus marker technique developed by Vos *et al*., (1995), combines in it the basic features of both, the classical hybridization based RFLPs and the PCR based approaches. The

procedure to generate AFLPs is also called selective restriction fragment amplification (SRFA), since it is based on the selective PCR amplification of a subset of restricted DNA fragments using the PCR procedure. AFLPs are reproducible biallelic markers (Jones *et al.*, 1997) that are distributed through the genome as restriction sites, and they allow estimates of relative genetic distances among individuals / species. The key feature of AFLP–PCR is its capacity for the simultaneous screening of many different DNA regions distributed randomly throughout the genome. To achieve high reliability of the screen, genomic DNA is prepared in an ingenious, but technically straightforward, way that combines the strengths of two methods, the replicability of restriction fragment analysis and the power of the PCR. In essence, AFLP methods allow the detection of polymorphisms of genomic restriction fragments by PCR amplification.

AFLP usually detected as the presence or absence of an amplified fragment and polymorphisms are arised in restriction site or selective nucleotide priming site sequences or due to insertion/deletion within amplified fragment (Ajmone-Marsa *et al.,* 1997 and 2002). AFLP-PCR products can be separated using simple agarose or polyacrylamide gel electrophoresis. AFLP markers have proved useful for assessing genetic differences among individuals, populations and independently evolving lineages, such as species. AFLP marker technology is a relatively cheap, easy, fast and reliable method to generate hundreds of informative genetic markers (Mueller and Wolfenbarger, 1999).

Several comparative studies using different molecular marker systems led to the conclusion that AFLP is the most efficient technique for detecting DNA polymorphism (Linn *et al.*, 1996; Roldan- Ruiz *et al.,* 2000). AFLP has been used to study genetic diversity and relationships in cattle (Ajmone-Marsan *et al.,* 1997 and 2002), goat (Ajmone-Marsan *et al.,* 2001), pigs (O` vilo *et al.,* 2000) and poultry (De Marchi *et al.,* 2005 and Gao *et al.,* 2007).

AFLP has not been extensively used in animals including poultry. AFLP markers are commonly used for large-scale evaluation of genetic diversity in farm animals, as a component of the management of animal genetic resources. AFLP markers are useful for such studies as they can be generated relatively simply; however, challenges in analysis arise from their dominant scoring and the low level of polymorphism of some markers. The main disadvantage of AFLP is the difficulty in identifying homologous markers (alleles), rendering this method less useful for

studies that require precise assignment of allelic states. Nevertheless, because of the rapidity and the ease with which reliable, high- resolution markers can be generated (Vos *et al.,* 1995).

Herbergs *et al.,* (1999) described the mapping of AFLP markers in chicken using a multicolour fluorescent detection system. Within the 57 *Eco*RI/*Taq*I primer pair combinations used a total of 475 polymorphic AFLP markers could be detected in a population consisting of four families with a total of 183 F_2 individuals. The number of AFLP polymorphisms detected per primer pair varied from 0 to 21, with an average of 8.5 AFLP markers per primer pair.

Knorr *et al.,* (1999) used amplified fragment length polymorphism (AFLP) technique to enhance marker density in the East Lansing reference chicken genome map, using a backcross family derived from a Red Jungle Fowl by White Leghorn mating with White Leghorn as the recurrent parent. They used 36 primer combinations and generated a total of 377 AFLP in the EL population. They mapped 204 AFLP markers and expand the overall map coverage by about 25%. In the backcross progeny they found JF-specific and WL-specific AFLP markers. In Jungle fowl they found 209 specific AFLP, out of this 10 (5%) could not mapped (e.g. shoulder bands). Of the 204 markers assigned, 199 JF-specific and five on the W chromosome.

De Marchi *et al.*, (2005) assessed genetic variation in four indigenous chicken breeds from the Veneto region of Italy using amplified fragment length polymorphism (AFLP) markers. Four indigenous Veneto chicken breeds (Ermellinata, Padovana, Pe´poi and Robusta) and a reference broiler line were included in the analysis. The three primer combinations revealed 188 bands; 70 of them were distinct AFLP polymorphisms (37%), with an average of 23.3 ± 1.7 markers per primer pair and a range from 21 to 25. The number of polymorphisms observed within breeds varied from 34 (broiler, with an average number per primer pair of 11.3 ± 0.5) to 43 (Padovana, with an average of 14.3 ± 2.6). Breed-specific markers were detected in each breed. The broiler line showed the highest number of diagnostic markers with four monomorphic bands. Ermellinata, Pe´poi and Robusta showed two breed-specific markers, and only one specific band was found for Padovana. The expected heterozygosity (He) did not differ significantly among the indigenous Veneto chicken breeds and the broiler line. Nei's standard genetic distance between pairs of breeds showed that the distance between the broiler line and the Pe´poi breed was greater

than the distances between the broiler line and the other three chicken breeds. Cluster analysis based on standard genetic distances between breeds indicated that the Padovana and Pe´poi breeds were closely related.

Mekchay *et al.*, (2005) used AFLP to assess the genetic diversity and specific marker between Thai native chickens and fast-growing broilers. Fifteen *Eco*RI/*Taq*I primer combinations were used to generate AFLP markers among 10 pooled DNA samples from chicken products essentially in carcass form that were ascribed as belonging to either slow (Thai native chicken) or fast-growing strains (broiler). Total 493 AFLP bands were detected of which 199 revealed as polymorphic bands. Phylogenic tree analysis was able to cluster separately Thai native chickens and commercial broiler chickens. Additionally, two AFLP fragments were identified as type-strains specific markers. With E-ACT / T-CAT primer combination, they found a band (270 bp) that was specific for slow-growing chickens, and another band (250 bp) that was specific for fast-growing chickens.

Gao *et al.*, (2007) used six AFLP primer combinations to detect genetic variation in 12 Chinese indigenous chicken breeds. The six primer combinations, giving, on average, 46.5 polymorphic markers detected per primer combination, generated a total of 279 polymorphic bands. Nine specific bands were produced in the pooled DNA of Jiuyuan black and Dongxiang black chickens. However, one specific band was produced in the pooled DNA of Wenchang and Xingyi bantam chickens. An unweighted-pair-group method using average linkages (UPGMA) cluster analysis revealed that the 12 chicken breeds could be divided into three groups. Genetic similarity coefficients and the UPGMA tree of the 12 chicken breeds were consistent with their breeding history as well as their geographical distribution. The genetic similarity coefficient between Qingyuan partridge and Xiayan chickens was the highest (0.860). These are both miniature and meat-type breeds of China.

2.2.2.1. Application of AFLP markers in other farm animals

Ajmone-Marsan *et al.*, (1997) described amplified fragment length polymorphism (AFLP) technology for DNA fingerprinting in cattle. The AFLP technology produces molecular markers through the high-stringency polymerase chain reaction (PCR)-amplification of restriction fragments that are ligated to synthetic adapters and amplified using primers, complementary to the adapters, which carry selective nucleotides at their 3' ends. While, for plants, the double digestion of

genomic DNA with *Eco*RI and *Mse*I is suggested, in mammals the enzyme combination *Eco*RI/*Taq*I produces clearer and more polymorphic AFLP patterns. In a sample of 47 Italian Holstein genotypes, 16 *Eco*RI/*Taq*I primer combinations identified 248 polymorphic bands in a species known for its low level of restriction polymorphism. Out of 248, 160 AFLP markers could be scored codominantly, while 88 markers could be scored only dominantly.

O'vilo *et al.,* (2000) used AFLP technique for the characterization of highly inbred Iberian pig breed genotypes and the detection of strain specific polymorphisms. They used 12 *Eco*RI/*Mse*I primer combinations to genotype animals belonging to two black hairless Iberian pig strains, Guadyerbas and Coronado. These amplification reactions allowed the detection of more than 1700 amplification products. Out of these 1733, only 106 (6%) were found polymorphic. Within those polymorphic bands, 26 were identified as strain-specific markers. 14 were specific for the Guadyerbas strain and 12 others for the Coronado strain.

Ajmone-Marsan *et al.,* (2002) used biallelic AFLP polymorphisms for the estimation of relative genetic distances of cattle individuals within or across breeds in a panel of 44 Italian Holstein-Friesian, 29 Italian Brown and 43 Maremmana animals. Four highly informative *Eco*RI/*Taq*I primer combinations generated 313 fragments and 106 polymorphic markers in the size range of 60-550 bp. 58 of the106 polymorphic markers (54.7%) were polymorphic in all three breeds and 28 (26.4%) in two breeds. They did not find breed-specific markers for any population. The G_{ST} index on the basis of allele frequencies assigned 88% of the total diversity to the diversity within the breeds.

Cameron *et al.*, (2003) used amplified fragment length polymorphic (AFLP) markers to discriminate between lines of pigs, divergently selected over seven generations for components of efficient lean growth rate. A total of 270 animals with 30 animals per line were genotyped for 239 polymorphic AFLP markers. Canonical variate analysis identified linear combinations of the AFLP marker scores that grouped animals by selection line with no overlap between selection lines. Cluster analysis of AFLP marker scores identified 10 groups of animals with 226 of the 270 animals clustered into nine groups, each consisting of animals from only one selection line. They concluded that AFLP marker genotyping, using the *Eco*RI and *Taq*I restriction enzymes, provided an effective means of discriminating between animals of different selection lines that have arisen from one base population.

2.2.3. Genetic differentiation and Gene flow estimation in chickens

Dai *et al.,* (2006) used five chicken populations (namely as New Yangzhou (NY-1), Rugao (HR-1), Jiangchun (HJ-2), Wan-Nan (HW-3) and the Cshiqishi (HC-4) chickens) to estimate Genetic differentiation and gene flow among populations by employing a suite of marker panel containing five carefully selected Micro satellite loci with 81 genomic DNAs isolated from the chicken's blood samples. The F_{IS} and F_{IT} - values generated varied for each locus across populations. They were – 0.1172 to 0.1815 and – 0.0908 to 0.2111 for F_{IS} and F_{IT}, respectively. The multi-populations F_{ST} ranged from 0.0082 (MCW4) to 0.0415 (ADL176). Using simple substitution common to population genetic studies, the gene flow depicted as Nm was between 5.7741 (ADL176) and 30.2378 (MCW4).

Cuc *et al.,* (2006) assessed genetic differentiation in H'mong chickens, a local breed in the mountainous areas of Northern Vietnam. A subset of thirty-six chickens from the three villages was genotyped at 29 microsatellite loci. The average inbreeding coefficient (F_{IS}) was 0.044, and the overall values of F_{IT} and F_{ST} were 0.069 and 0.026, respectively. They suggested that the overall genetic differentiation observed in this study is low indicating little genetic effects of drift or mutation in the subpopulations.

Musa *et al.,* (2007) used a total of 243 individuals from RJF (*Gallus gallus Spadiceus*), Rugao, Anka, Wenchang and Silikes chicken populations for polymorphism analysis in functional apo VLDL-II gene by RFLP and SSCP markers. The averages of H_T, H_S and G_{ST} across all loci were 0.3417, 0.241 and 0.296, respectively. The estimates of G_{ST} were further used to calculate the gene flow (Nm). The Nm was ranged from 0.5884 (at locus VLDL-17) to 3.1454 (at locus VLDL-6) with an average 1.1890 across loci.

Kanginakudru *et al.,* (2008) analysed 76 Indian birds that included 56 *G. g. murghi* (RJF), 16 *G. g. domesticus* (domestic chicken) and 4 *G. sonneratii* (Grey JF) using 11 microsatellite markers. Microsatellite marker analysis of Indian birds indicated an average F_{ST} of 0.126 within *G. g. murghi* (RJF), and 0.154 within *G. g. domesticus* while it was more than 0.2 between the two groups. The microsatellite-based phylogenetic trees showed a clear separation of *G. g. domesticus* from *G. g. murghi*, and *G. sonneratii*. The pairwise F_{ST} value was very low within *G. g. murghi*

when compared to *G. g. domesticus*. The average F_{ST} value was more for *G. sonneratii – G. g. domesticus* combination than for *G. sonneratii-G. g. murghi* or *G. g. murghi – G. g. domesticus* combinations.

Bao *et al.,* (2009) evaluated genetic differentiation and gene flow between RJF and 14 Chinese indigenous chicken breeds using 29 microsatellite markers. Genetic differentiation was examined by fixation indices F_{IT}, F_{ST} and F_{IS} for each locus. The average of genetic differentiation among populations, measured as F_{ST} value, for the 29 loci varied from 0.101 (MCW0020) to 0.319 (MCW0081), with a mean of 0.164 (16.4%). The global deficit of heterozygotes across populations (F_{IT}) amounted to 18%. An overall significant deficit of heterozygotes (F_{IS}) of 2% occurred at the analyzed loci because of inbreeding within populations. Reynolds' distance values varied between 0.0478 (Xiaoshan chicken – Luyuan chicken pair) and 0.3353 (red jungle fowl – Henan game chicken pair). The Nm value ranged from 0.4967 (between red jungle fowl and Gushi chicken pair) to 5.1033 (between Xiaoshan chicken and Luyuan chicken pair). Most of Nm values between pairs of breeds were below 2.0.

Materials and Methods

Material and Methods

3.1. Resource populations

A total of 76 birds of four populations namely Red Jungle Fowl, White Leghorn, Aseel and Red Cornish were used. In this analysis we included 20-20 birds from Red Jungle Fowl (RJF) and White Leghorn population (WLH) each and 18-18 birds of Aseel (AS) and Red Cornish (RC) populations each with almost half-half number of male and female birds. Each population was represented as a specific type likewise RJF- wild type, WLH- egg type, AS- Indian native chicken breed as well as game type and RC- meat type.

3.2. Extraction of genomic DNA

Blood samples of about 0.5 ml were collected from Jugular vein in 1.5 ml-eppendorf tube containing EDTA from each bird of RJF and other three domestic chicken populations. All the blood samples were stored at –20°C till further processing. The high molecular weight genomic DNA was isolated using the following simple method. To 50 µl of blood, 700 µl of lysis buffer (10 mM Tris. HCl, 100 mM NaCl, 1 mM EDTA, pH: 8.0 and 0.5% SDS) containing 60 µg of proteinase K (20 mg/ml) was added. The mixture was vigorously vertexed and incubated at 37°C for 10-12 hours with gentle shaking. The DNA was purified by extracting with equal volume of phenol, phenol-chloroform and chloroform-isoamylalcohol (24:1). The genomic DNA was precipitated by adding 1/10 volume of 3 M sodium acetate and two volumes of ice chilled ethanol and centrifuged for 5 minutes at 14000 g. DNA pellet was then washed with 70% ethanol and air-dried. DNA pellet was dissolved in a minimum volume of TE buffer (10 mM Tris, 1mM EDTA, pH- 8.0).

3.2.1. DNA Quantification

DNA Quantification was done using Nanodrop spectrophotometer (Thermo Scientific, USA) by taking only 1 µl of DNA. After the complete dissolution of DNA, its optical density at 260 and 280 nm was determined. Taking the ratio of optical

density at 260 and 280 nm checked the purity of DNA. The DNA samples having O.D. ratio between 1.7 and 1.9 have been used for the study.

It is known the one O.D. unit at 260 nm equals 50 µg/ml of pure, double stranded DNA. Therefore, the concentration of DNA samples was calculated by the following formula

Concentration (µg/ml) = O.D. at 260 nm x Dilution ratio x 50

3.2.2. Examination of Quality of DNA

The quality of genomic DNA was also examined by horizontal electrophoresis of DNA samples on 1.0% agarose gel. Loading samples were prepared by adding 2.0 µl of DNA, 2 µl of 6X Bromophenol blue and 5 µl of distilled water. Electrophoresis was performed at 3 V/cm (Max. 5 V/cm of gel) for 1 to 2 hrs. Finally the gel was examined under UV light. The good quality DNA samples having intact DNA bands without any smearing was selected for further analysis.

The genomic DNA from each bird was diluted into two sets of concentration. First set had the concentration of 25-30 ng/µl and was used for Microsatellite marker analysis while second set had the concentration of 50 ng/µl and was used for AFLP marker analysis.

3.3. Microsatellite Marker Analysis

3.3.1. Selection of Markers

A set of 15 tetra-nucleotide (LEI series) microsatellite primer pairs (McConnell *et. al.,* 1999) and 10 di-nucleotide (9 of ADL and one of MCW series) microsatellite primer pairs (Groenen *et al.,* 2000) were used for genotyping of 76 birds of 4 populations. The relevant information about all microsatellite loci is shown in **Table- 3.1.**

3.3.2. Genotyping on horizontal gel electrophoresis using metaphor agarose

23 microsatellite markers were used for genotyping on metaphor agarose gel electrophoresis.

3.3.2.1. PCR reaction setup

Initially varying various parameters like as annealing temperature, $MgCl_2$ and cycle number standardized the amplification conditions. Finally the following amplification conditions were used.

Table 3.1. Details of 25 Microsatellite markers used in present study

Locus	Primer sequence		Repeat motifs	Ta (°C)	Map locations
ADL034	(F) AACCTAAAAACTCCTGCTGC	(R) GGGAACCTGTGGGCTGAAAG	$(CA)_{11}$	55ºC	E47W24
ADL120	(F) GCATTCCAACTCCCCTTTGG	(R) ACCAGATATAACAGTCCTCT	$(GT)_{9}$	55°C	2
ADL158	(F) TGGCATGGTTGAGGAATACA	(R) TAGGTGCTGCACTGGAAATC	$(CA)_{12}$	55ºC	E29C09WO9
ADL209	(F) GGTTAGCTCCCTCCTTCCAG	(R) TCACTCCAGCTTGAGACAGG	$(CA)_{20}$	55ºC	E29C09WO9
ADL254	(F) CAGGCTGGAAGCAATAAATG	(R) CACTCATCCCACAACCACTG	$(TG)_{13}$	55ºC	28
ADL265	(F) GCCTTCTGAAGGTAAGTAGC	(R) TGTCAGCAGAGCAGCAATAC	$(GT)_{46}$	55ºC	4
ADL270	(F) TGGGGTTGGGTTGGTTTTTA	(R) CCCTGGCAGTTGGTTATTCT	$(GT)_{20}$	55ºC	2
ADL327	(F) GCANNACCAGTCCATCACGA	(R) AGAGGGTAAGAAATCCTGCTG	$(CA)_{21}$	55ºC	3
ADL331	(F) CCAAACTCCCCCAAGCATTC	(R) GGGAGCCTTCACAAGACAAA	$(CA)_{8}$	55ºC	4
LEI192	*(F) TGCCAGAGCTTCAGTCTGT	(R) GTCATTACTGTTATGTTTATTGC	$(T)_{8}CCCTTT(CTTT)_{11}$	55ºC	6
LEI194	(F) TCCTTGGCATGTACATATGA	(R) ACTGCATGTTCTTTGATAGGC	$(TTTC)_{15}(T)_{10}$	58ºC	1
LEI209	(F) AATTTGGTGTCATACCTCTCC	(R) GACTTTCCAGTGTCTCGTTTAG	$(CTTT)_{22}$	58ºC	1
LEI212	(F) TTTGCCAATCCCTATTGAGC	(R) TTTTCATATTTGTGGCGTGC	$(CTTTCTTC)_{5}$	58ºC	6
LEI214	(F) TGCCTCGTCTTACTGAGTGA	(R) GATCAAGCACTGTATTTTATTC	$(CTGT)_{7}(CTTT)_{8}$	58ºC	E30C14W10
LEI217	(F) GATGACTGAGAGAAATAACTTG	(R) AAATTACTGAGGCACAGGAG	$(CTTT)_{31}$	58ºC	1
LEI221	(F) AATTTGGTGTCATACCTCTCC	(R) GACTTTCCAGTGTCTCGTTTAG	$(CTTT)_{21}$	59ºC	1
LEI228	(F) GCTGGGTTATTTCAATATGTGG	(R) AGCGTACCTGATAATGATGAGC	$(CTTT)_{6}$	56ºC	2
LEI229	(F) CAGTTCCAAAGGCAAGTCAGG	(R) CGGTTAGGTTTGAAGTGCATGG	$(CTTTCCTTT)_{18}$	56ºC	W/Z
LEI232	(F) CAGGCTGGAAGCAATAAATG	(R) CACTCATCCCACAACCACTG	$(CTTT)_{4}(CT)_{2}(CTTT)_{17}$	55ºC	1
LEI234	(F) ATGCATCAGATTGGTAATCAA	(R) CGTGGCTGTGAACAATATG	$(TTTC)_{18}$	58ºC	2
LEI237	(F) GTTAAGTGTTCTCTGATGTAGC	(R) CTTCAACTATAAAGCATAGCTG	$(CTTT)_{17}$	58ºC	2
LEI243	(F) TTCAAATCTGTCACTGGAAAGG	(R) CAGGGTGCATGTGTATCATACC	$(TTTC)_{26}(T)_{6}$	55ºC	2
LEI246	(F) TTGCACTGAGACCAAATGTC	(R) CATAGATTTTCCTTAGTAGGTAACTTG	$(CTTT)_{28}$	55ºC	1
LEI248	(F) TTTGAAAGTGACCATGATTCG	(R) AAGCAGTTTCCAAGCTAAGAAC	$(TTTC)_{25}$	51ºC	2
MCW111	*(F) GCTCCATGTGAAGTGGTTTA	(R) ATGTCCACTTGTCAATGATG	$(CA)_{7}$	55ºC	1

*6-FAM labeled

PCR Amplification was carried out in a final volume of 25 µl reaction mixtures in 0.2 ml thin wall PCR tubes. Each PCR tube containing 30-50 ng genomic DNA, 1.5 mM $MgCl_2$, 1X Reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH-8.8, 0.1% Triton X-100, 0.01% gelatin), 200 µM of each dNTP (dATP, dGTP, dCTP and dTTP), 1U of *Taq* DNA polymerase enzyme and 5 pmol of each forward and reverse primer.

3.3.2.2. PCR amplification conditions

The amplification for all primers was carried out in a thermocycler (Eppendorf -Germany). Protocol for each PCR reaction consisted of an initial denaturation at 94°C for 3 min. followed by 34 cycles of PCR, each cycle consisting of 30 s at 94°C, 45 s at 55 –60°C* (*variable) and 1.30 min at 72°C, and followed by a final extension step of 10 min at 72°C.

3.3.2.3. Resolution and documentation of Microsatellite alleles

After the completion of PCR reaction, five micro liters of tracking dye/ stop dye (80% formamide, 50 mM Tris-Hcl (pH-8.8) 1 mM EDTA, 0.1% bromophenol blue and xylene cyanol) was added and the samples were stored at 4°C till further use.

The amplification products from the microsatellite markers were resolved on 3.5% metaphor agarose gel. The gels were stained with ethidium bromide and scanned and photographed by a Phosphor-imager (FLA-5100, Fluorescent / Radioisotope Science Imaging System, Fugifilm, Japan).

Molecular sizes of various alleles of Microsatellite markers were estimated by using 20 bp DNA ladder (Bangalore Genei) as molecular size marker. The alleles at different microsatellite locus were sized using computer software.

3.3.3. Genotyping on capillary electrophoresis

Two microsatellite marker (LEI192 and MCW 111) labeled at 5' with 6-carboxyfluorescein (6-FAM) dye were used for genotyping on Automated DNA Sequencer (3130xl Genetic Analyzer from Applied Biosystems).

3.3.3.1. PCR reaction setup

PCR Amplification for Genetic Analyzer samples were carried out in a final volume of 20 µl reaction mixtures in 0.2 ml thin wall PCR plates. Each PCR well containing 25-50 ng genomic DNA, 10µl of *AmpliTaq* Gold® PCR Master Mix (contents as per supplied by Applied Biosystems) and 2.5 pmol of each 5'-FAM labeled forward and unlabeled reverse primer.

3.3.3.2. PCR amplification conditions

The amplification for all primers was carried out in a thermocycler (Eppendorf -Germany). Protocol for each PCR reaction consisted of an initial denaturation at 94°C for 3 min. followed by 34 cycles of PCR, each cycle consisting of 30 s at 94°C, 45 s at 55 –60°C* (*variable) and 1.30 min at 72°C, and followed by a final extension step of 10 min at 72°C.

3.3.3.3. Resolution and documentation of microsatellite alleles

The amplified products were first tested on 2% agarose gel to confirm amplification. Afterthat 0.5 µl of PCR product was mixed with 10.20 µl of Hi-Di™ Formamide (supplied by Applied Biosystems) and 0.30 µl of GeneScan –500 LIZ® internal Size Standard (supplied by Applied Biosystems) was included in the loading samples. The samples were denatured at 94°C for 3 min followed by immediately placed on ice before loading the sample on 3130xl Genetic Analyzer, fully automated capillary based system.

Data on genetic analyzer was collected by using Data Collection Software® (Applied Biosystems). Allele sizes were estimated using Genemapper® Software version 4.0 (Applied Biosystems).

3.3.4. Statistical Analysis

The microsatellites data obtained with the RJF and other three chicken populations were used for the measurement of genetic parameters of the populations. PowerMarker computer software were employed to calculate number of alleles (N); allele frequencies (F); observed heterozygosity (H_O); gene diversity (expected heterozygosity) (H_E) ; Polymorphism Information Content (PIC) within populations and across all populations at each locus. PowerMarker genotype information always used to estimate all parameters. Genetic distances and other phylogenetic relationships between populations were also calculated. Genetic distances and other phylogenetic relationships between populations in PowerMarker software is based on frequencies of alleles (Liu *et al.*, 2005). Frequency based UPGMA dendrograms were constructed by MEGA 3.0 Software (Kumar *et al.,* 2004) embedded in PowerMarker. The identification of breed-specific (population-specific) alleles for each breed was done manually with respective allele frequencies calculated by PowerMarker software. POPGENE software ver 1.31 (Yeh *et al.*, 1999) was employed to calculate

genetic differentiation/F-statistic and gene flow between populations across loci and across populations at each locus.

3.3.4.1. Allele frequencies (F)

The alleles at different microsatellite locus were sized using computer software. The allele frequency was estimated as its proportion to the total no of loci (2n), where n is the number of individuals genotyped at that microsatellite locus.

3.3.4.2. Observed and expected heterozygosity

Observed heterozygosity at a microsatellite locus was measured as proportion of the heterozygous individuals at that microsatellite locus as follows

Observed heterozygosity (Ho) = H / T

Where H is the number of heterozygotes individuals at a locus and T is the total number of individuals genotyped at that locus.

Expected heterozygosity (H_E) at a locus was estimated using an unbiased estimator

$$H_E i = (2N/(2N-1)\ \{\ 1 - \sum_{j=1}^{l} P_j^{\ 2}\}$$

Where P_j is the frequency of j^{th} allele at i^{th} locus with l alleles in a population, and N is the number of individuals genotyped at ith locus.

3.3.4.3. Polymorphic Information Content (PIC)

The PIC was also calculated using microsatellite allelic frequencies as follows

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i^2 p_j^2$$

Where, n=the number of alleles, P_i and P_j are the frequencies of i^{th} and j^{th} alleles at a locus in a population, respectively (Botstein *et al.*, 1980).

3.3.4.4. Frequency-based genetic distance

The shared allele distance D_{SA} (Chakraborty and Jin, 1993), is defined as:

$$D_{SA} = \frac{1}{m} \sum_{J=1}^{m} \sum_{i=1}^{a_j} \min (p_{ij}, q_{ij})$$

Here p_{ij} and q_{ij} be the frequencies of *i* th allele at the *j* th locus in populations X and Y respectively, while a_j is the number of alleles at the *j* th locus, and *m* is the number of loci examined.

3.3.4.5. Genetic differentiation

Genetic differentiation was examined by fixation indices F_{IS}, F_{IT} and F_{ST} for each locus across populations and among populations following the *F*-statistics of Wright (1965) modified by Hartl and Clark (1989). The measures of F_{IS}, F_{IT} and F_{ST} are related to the amount of heterozygosity at various levels of population structure. Together, they are called F- statistics, and are derived from F, the inbreeding coefficient.

$$F_{IS} = \frac{H_S - H_I}{H_S}, \quad F_{ST} = \frac{H_T - H_S}{H_T}, \quad F_{IT} = \frac{H_T - H_I}{H_T},$$

where, H_I be the actual heterozygosity in individuals within subpopulations, H_S be the expected heterozygosity within subpopulations, H_T be the expected heterozygosity in the combined populations.

$$(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$$

where, F_{IS} is an estimate of variation within population, F_{IT} is the overall inbreeding coefficient of an individual relative to the total population and F_{ST} is an estimate of variation due to differences among populations.

3.3.4.6. Gene flow estimations

Gene flow between populations, defined as the number of reproductively successful migrants per generation (Nm). Then estimate was based on the relationship of Nm with F_{ST}.

$$Nm = 0.25(1 - F_{ST})/F_{ST}$$

Where N= effective population size, m= migration rate and F_{ST} is calculated as mean over loci.

3.4. AFLP marker analysis

The AFLP assay was performed as previously described by Vos *et al.,* (1995); Vuylsteke *et al.,* (2007) with some modifications use of *Taq*I restriction endonuclease enzyme instead of *Mse*I, which is recommended for poultry (Knorr *et al.,* 1999). AFLP primers were named '+0' when they have no selective bases (only the core,

enzyme-specific and restriction-site remnant sequence), '+1' when they have a single selective base, '+2' when they have two selective bases, '+3' when they have three selective bases and so on. The sequence of *Eco*RI and *Taq*I adapters and primers for pre-amplification and selective amplification step is shown in **Table –3.2.**

3.4.1. Template Preparation

To prepare an AFLP template, the isolated genomic DNA was digested with two-restriction endonucleases- a rare cutter and a frequent cutter (*Eco*RI and *Taq*I). The frequent cutter was used to generate fragments that were in the range of 50-500 bp, easily resolvable by electrophoresis.

3.4.1.1. Restriction Digestion of DNA

About 400 ng of genomic DNA (10 µl) was incubated with 15 µl of *Taq*I restriction digestion mix (containing 5U *Taq*I RE and RL buffer) for *Taq*I restriction digestion for 1 h at 65°C and subsequently with 15 µl of *Eco*RI restriction digestion mix (containing 5U *Eco*RI RE and RL buffer) for *Eco*RI restriction digestion for 1 h at 37°C.

3.4.1.2. Ligation of Adapters

Adapters were ligated to the restriction fragments by addition of a ligation mix (10 µl) containing *Eco*RI adapter (5 pmol) and *Taq*I adepter (50 pmol) with 1U T4 DNA ligase. The reactions were continually incubated for another 3 h at 37°C. After ligation, reaction mixture (50 µl) was diluted to 200 µl with TE (10:1) buffer. This was served as template for the pre-amplification reaction.

3.4.2. Pre-amplification of template DNA

Amplification of restriction fragments was performed in two consecutive PCR rounds (pre-amplification and selective- amplification). The first round or Pre-amplification reaction was performed with primers complementary to the adapters *Eco*RI and *Taq*I with one selective nucleotide at 3' end to reduce the number of bands in second round or selective amplification. The primer combination used in this pre-amplification step was: *Eco*RI + 1nt. (E+**1**) and *Taq*I + 1nt. (T+**1**). Pre-amplification increases the amount of template and also helps to ensure that the final amplification will be completely selective. The pre-amplification was carried out in a final volume of 50 µl containing 5 µl of diluted ligation product (from step-3.4.1.2.) as template, 15 pmol *Eco*RI primer, 15 pmol *Taq*I primer, 200 µM of dNTP mix (50 µM of each dNTP), 2.5 mM $MgCl_2$, 1U *Taq* polymerase and 1X PCR buffer.

Table 3.2. Sequences of adapters and primers used in AFLP analysis

Specifications	Name	5'-3' sequences
Adaptors		
*Eco*RI adaptors	Top strand	5'-CTCGTAGACTGCGTACC
	Bottom strand	5'-AATTGGTACGCAGTCTAC
*Taq*I adapters	Top strand	5'-GACGATGAGTCCTGAC
	Bottom strand	5'-CGGTCAGGACTCAT
Primers		
*Eco*RI primer +0	E + 0	5'-GACTGCGTACCAATTC
*Taq*I primer +0	T + 0	5'-GATGAGTCCTGACCGA
Pre-amplification primers		
*Eco*RI primer +1nt	E + 1	5'-GACTGCGTACCAATTC**A**
*Taq*I primer +1nt	T + 1	5'-GATGAGTCCTGACCGA**A**
***Eco*RI Selective amplification primers**		
*Eco*RI primer +3nts	E01	5'-GACTGCGTACCAATTC**ATA**
*Eco*RI primer +3nts	E02	5'-GACTGCGTACCAATTC**AGC**
*Eco*RI primer +3nts	E03	5'-GACTGCGTACCAATTC**ACA**
*Eco*RI primer +3nts	E04	5'-GACTGCGTACCAATTC**AGT**
***Taq*I Selective amplification primers**		
*Taq*I primer +3nts	T01	5'-GATGAGTCCTGACCGA**AAT**
*Taq*I primer +3nts	T02	5'-GATGAGTCCTGACCGA**ACT**
*Taq*I primer +3nts	T03	5'-GATGAGTCCTGACCGA**AAG**
*Taq*I primer +3nts	T04	5'-GATGAGTCCTGACCGA**AGC**
*Taq*I primer +3nts	T05	5'-GATGAGTCCTGACCGA**ATG**
*Taq*I primer +3nts	T06	5'-GATGAGTCCTGACCGA**ATGT**

Thermocycler profile was as follows: 30 s at 94°C, 1 min at 56°C and 1 min at 72°C for 25 cycles, followed by a final extension step of 5 min at 72°C. An aliquot of the pre-amplification reaction product was diluted 20-fold with T:E (10:1) buffer. These diluted pre-amplified reaction products were served as templates for the selective amplification.

3.4.3. Selective Amplification

The second round or selective amplification was performed using primers having the same sequence as those used in Pre-amplification but with three to four selective nucleotide at 3' end. A total of 20 *Eco*RI and *Taq*I primer combinations with 3 to 4 selective nucleotides were used for genotyping of all 76 birds. Selective PCR amplifications were carried out in a final volume of 20 µl reaction mixtures containing 5 µl of diluted pre-amplified reaction products (from step- 3.4.2.), 1 pmol *Eco*RI primer +3nts, 6 pmol *Taq*I primer +3/4 nts, 2.5 mM $MgCl_2$, 200 µM of each dNTP (dATP, dGTP, dCTP and dTTP), 0.6 U *Taq* polymerase and 1X Reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH-8.8, 0.1% Triton X-100, 0.01% gelatin).

A touchdown-PCR was performed, starting with 2 cycles of 30 s at 94°C, 30 s at 66°C and 60 s at 72°C, reducing annealing temperature by 2°C in next four steps of 2 cycles each. The PCR proceeded with 25 cycle of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C, followed by a final extension step of 5 min at 72°C.

The pre- and Selective-amplification reactions were performed in a thermocycler (Eppendorf, Germany).

3.4.4. Resolution and documentation of AFLP bands

After the completion of PCR reaction, five micro liters of tracking dye/ stop dye (80% formamide, 50 mM Tris-Hcl (pH-8.8) 1 mM EDTA, 0.1% bromophenol blue and xylene cyanol) was added and the samples were stored at 4°C till further use.

The amplification products from the AFLP markers were resolved on 3.5% metaphor agarose gel. The gels were stained with ethidium bromide and scanned and photographed by a Phosphor-imager (FLA-5100, Fluorescent / Radioisotope Science Imaging System, Fugifilm, Japan).

Molecular sizes of AFLP markers were estimated by using 20 bp DNA ladder (Bangalore Genei) as molecular size marker. The scorable bands at different AFLP locus were sized using computer software.2

3.4.5. Statistical Analysis

Only intense and unambiguous AFLP bands were manually scored as dominant markers in binary matrices using values 1 and 0 (indicating band presence and absence respectively). Each band was treated as a separate putative locus with two alleles. Monomorphic loci had only one allele (present), while polymorphic loci had both (present and absent) alleles. Only loci with clearly amplified bands were used for data analysis. The AFLP data obtained with the RJF and other three chicken populations were used for the measurement of genetic parameters of the populations. POPGENE software ver 1.31 (Yeh *et al.*, 1999) was employed to calculate number of polymorphic band, level of polymorphism, Nei's (1973) gene diversity (h), Shannon's Information index (I) within populations and across all populations, coefficient of genetic differentiation (G_{ST}) and Gene flow among populations as per Nei's (1987) and also in calculation of Nei's (1972) original measures of genetic identity and genetic distance among populations. Relationships among individuals of each population and between wild and domestic chicken populations were evaluated using a dendrogram based on Nei's (1972) genetic distance. It was generated by the UPGMA (unweighted pair group method for arithmetic means) cluster analysis method by MEGA 3.0 Software (Kumar *et al.*, 2004). The identification of breed-specific (population-specific) alleles for each breed was done manually with respective allele frequencies calculated by POPGENE software ver 1.31software.

3.4.5.1. Polymorphism

The level of polymorphism (percentage of polymorphic bands) was calculated across all populations as well as for each population. To estimate the proportion of polymorphic loci (P) for a population where a number of loci have been collected, the following equation is used:

$$P = \frac{x}{m}$$

Where, x is the number of polymorphic loci in a sample of m loci. A locus is considered to be polymorphic if there are at least to individuals differ at this locus.

3.4.5.2. Nei's (1973) gene diversity (h)

Nei (1973) called this measure as gene diversity or genetic diversity.

$$h = 1 - \sum P_i^2$$

Where, Pi is the population frequency of each allele (1 and 0) at locus *i*. The average genetic diversity is then calculated as the average of this quantity across all loci studied.

3.4.5.3. Shannon's Information Index (I)

Shannon's (1949) information index is a measure of gene diversity.

$$I = -\sum p_i \ln (p_i)$$

Here p_i be the frequencies of *i* th allele at the given locus in a population and ln is the natural logarithm.

3.4.5.4. Nei's 1972 standard genetic distance

The standard genetic distance of Nei's (72) is one of the most commonly used genetic distances. For populations X and Y with r loci and m alleles per locus, the standard genetic distance is defined as:

$$D_S = -\ln (J_{XY}) / \sqrt{J_{XX} J_{YY}}$$

where,

$$J_{XY} = \sum_{i=1}^{m} \sum_{j=1}^{r} (x_{ij} \cdot y_{ij}) / r,\ J_{XX} = \sum_{i=1}^{m} \sum_{j=1}^{r} x_{ij}^2 / r,\ J_{YY} = \sum_{i=1}^{m} \sum_{j=1}^{r} y_{ij}^2 / r$$

x_{ij} is the frequency of the i^{th} allele at the j^{th} locus in population X, and y_{ij} is the frequency of the i^{th} allele at the j^{th} locus in population Y.

3.4.5.5. Coefficient of genetic differentiation (G_{ST}) and Gene flow (Nm) among populations

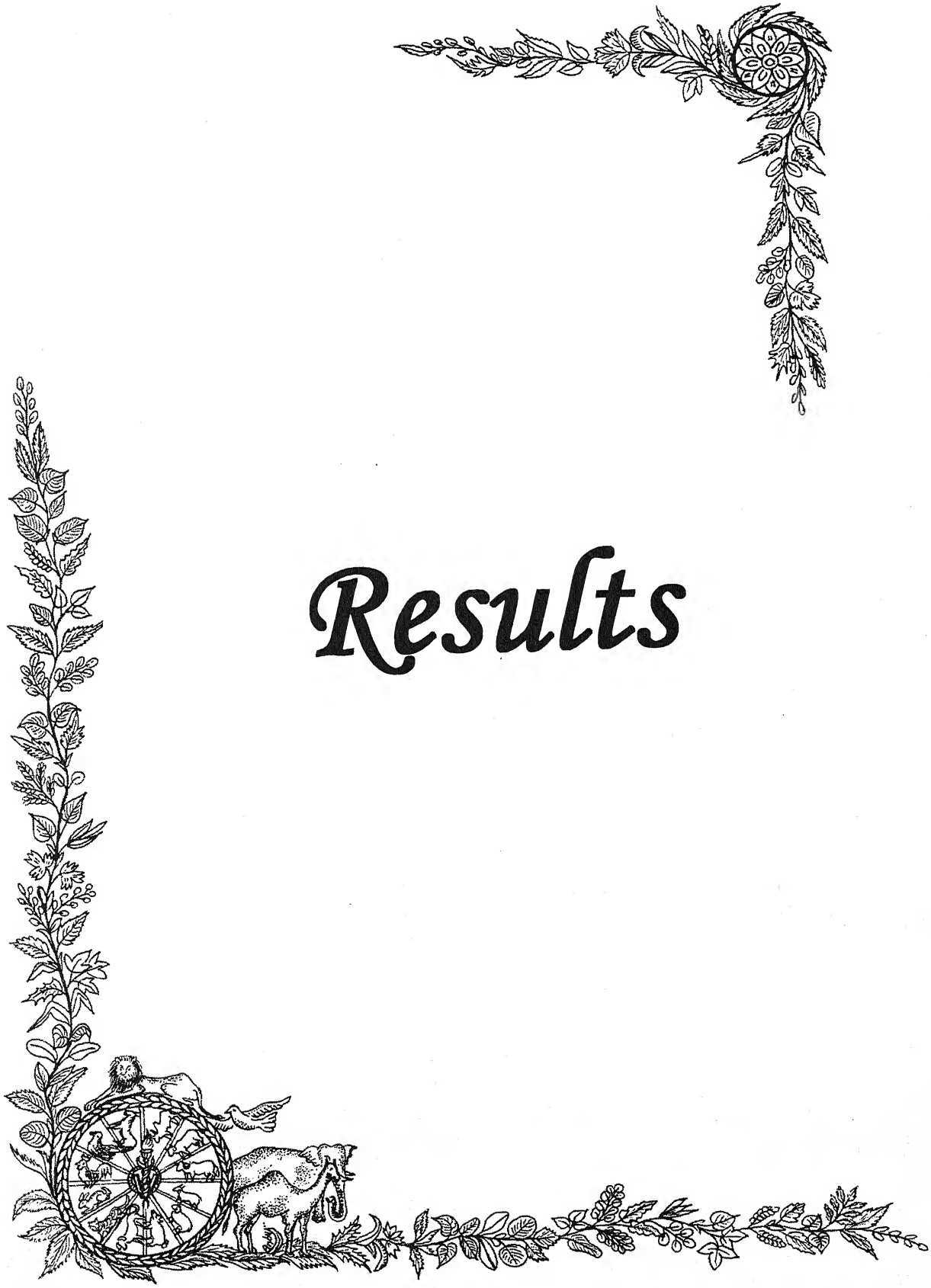
Coefficient of genetic differentiation (G_{ST}) and gene flow were estimate as per Nei's (1987).

$$G_{ST} = \frac{H_T - H_S}{H_T}$$

here H_T : Total genetic diversity, H_S : Genetic diversity within population, G_{ST} : gene diversity among populations (Coefficient of genetic differentiation).

Moreover, Gene flow (Nm) was estimated from G_{ST}.

$$Nm = \frac{0.5\,(1-G_{ST})}{G_{ST}}$$



Results

Results

4.1. Microsatellite marker analysis

4.1.1. Genetic diversity analysis

A total of 15 tetra-nucleotides and 10 di-nucleotides polymorphic microsatellite markers were used to genotype the resource population. The Number of alleles (N) and allelic size range in red jungle fowl, domestic chicken breeds and across all the populations at different microsatellite locus have been presented in **Table- 4.1,** while the respective Expected Heterozygosity (H_E), Observed Heterozygosity (Ho) and Polymorphic Information Content (PIC) in these populations have been presented in **Table- 4.2**. The microsatellite allelic profiles in different population at these loci are shown in **Fig 4.1 to 4.25**.

4.1.1.1. Microsatellite ADL034

For ADL034 microsatellite, the number of alleles ranged from 6 in RC to 13 in AS, however across the populations total number of alleles was 18. The size range across the population was 113 bp to 159 bp. Expected heterozygosity was moderate to high and ranged from 0.6194 to 0.8596 in different populations, while the values of observed heterozygosity (Ho) were observed higher in AS and RC in comparison to RJF and WLH. PIC values were from 0.5752 to 0.8454 in different populations. The estimates H_E, H_O and PIC were 0.9000, 0.5075 and 0.8924 respectively across the populations.

4.1.1.2. Microsatellite ADL120

At ADL120 locus, 1-8 alleles were observed in different populations, while across the population, total numbers of alleles were 12. The size range across the populations was 146-192 bp. This locus was monomorphic in White leghorn and Red Cornish populations. The highest number of 8 alleles was observed in Aseel followed by 4 alleles in RJF. Expected heterozygosity, observed heterozygosity and PIC value was measured higher in Aseel as compared to the RJF at this locus. The observed heterozygosity at this locus was found minimum (0.1486) across the populations.

4.1.1.3. Microsatellite ADL158

For ADL158 marker, 6-10 alleles were found in different populations, while across the population, total numbers of alleles were 13 and the allelic size range was from 172 bp to 208 bp. The expected heterozygosity was high (0.7647-0.8407), while the observed heterozygosity was comparatively low (0.3529 to 0.4737) in different populations. The values of PIC were high and ranged from 0.7332 to 0.8222 in different populations. The estimates H_E, H_O and PIC were 0.8909, 0.4384 and 0.8808 respectively across the populations.

4.1.1.4. MicrosatelliteADL209

At ADL209 locus, 4-11 alleles were found in different populations, while across the population, total numbers of alleles were 15 and the allelic size range was from 132 bp to 178 bp. The expected heterozygosity was found high and ranged from 0.6403 to 0.8472, while the observed heterozygosity was 0.3529 to 0.8333 in different populations. At this locus, observed heterozygosity within RC was found highest (0.8333) as compare to Ho estimated in RC on other loci. The values of PIC were ranged from 0.5738 to 0.8322 in different populations. The estimates H_E, H_O and PIC were 0.8783, 0.5000 and 0.8661 respectively across all the populations.

4.1.1.5. Microsatellite ADL254

For ADL254 marker, 8-18 alleles were found in different populations, while across the population, total numbers of alleles were 24 and the allelic size range was from 126 bp to180 bp. Expected heterozygosity was comparatively high (0.8438 to 0.8935). The observed heterozygosity was moderate to high and ranged from 0.4000 to 0.8000 in different populations. PIC values were high with more or less similar values and ranged from 0.8253 to 0.8666 in different populations. Within AS population, the highest value of expected heterozygosity and PIC (0.8935 and 0.8855, respectively) were observed at this locus as compare to these estimates on other loci. The estimates H_E, H_O and PIC were 0.9223, 0.5857 and 0.9172 respectively across all the populations.

4.1.1.6. Microsatellite ADL265

At ADL265 locus, 4 to 8 alleles were found in different populations; while across all the populations total number of alleles was 15 with the allelic size range between 100 bp to 144 bp. Expected heterozygosity was comparatively high (0.6593 to 0.7825), while the observed heterozygosity was variable and ranged from 0.1053 to

0.8000 in different populations. PIC values were moderately high and ranged from 0.6217 to 0.7493 in different populations. In RJF, low heterozygosity was observed in comparison to other chicken breeds. These estimates were 0.8858, 0.4400 and 0.8753, respectively across the populations. At this locus, observed heterozygosity within WLH population was found highest (0.8000) as compare to Ho estimated in WLH on other loci.

4.1.1.7. Microsatellite ADL270

For ADL270, the number of alleles ranged from 6 in RJF to 9 in AS, however across all the populations total number of alleles was 17. The size range across the population was 86 bp to 132 bp. Expected heterozygosity was found high and ranged from 0.6350 to 0.8443 in different populations. The observed heterozygosity was ranged from 0.2500 to 0.5882, while the PIC values were ranged from 0.6115 to 0.8258 in different populations. The estimates H_E, H_O and PIC were 0.8401, 0.4028 and 0.8266 respectively across all the populations.

4.1.1.8. Microsatellite ADL327

For locus ADL327, the number of alleles ranged from 6 in RJF to 8 in RC, however across the populations total number of alleles was 17. The size range across the population was 98 bp to 134 bp. Expected heterozygosity was found high and ranged from 0.5895 to 0.7145 in different populations. The observed heterozygosity was found moderate to high and ranged from 0.2105 to 0.7368, while the PIC value was ranged from 0.5610 to 0.6700 in different populations. The estimates H_E, H_O and PIC were 0.8862, 0.3973 and 0.8754 respectively across all the populations.

4.1.1.9. Microsatellite ADL331

For ADL331, marker, the number of alleles ranged from 6 in RJF to 13-13 in AS and RC each, while across the population, total number of alleles was 21 and the allelic size range was from 151 bp to 197 bp. Expected heterozygosity was ranged from 0.6055 to 0.8735. The observed heterozygosity was comparatively low to high and ranged from 0.2353 to 0.5556 in different populations. PIC value was ranged from 0.5706 to 0.8621 in different populations. Within WLH population, the highest value of expected heterozygosity (0.8675) and PIC (0.8534) were found at this locus as compare to these estimates on other loci. The estimates H_E, H_O and PIC (0.8735, 0.5556 and 0.8621 respectively) in AS population were found higher in comparison to

other chicken breeds at this locus. The estimates H_E, H_O and PIC were 0.9159, 0.3836 and 0.9100 respectively across all the populations.

4.1.1.10. Microsatellite LEI192

At locus LEI192, 16 alleles were present across the population in size range of 255 bp to 465 bp; while number of alleles in different populations was comparatively lower i.e. 3-8 alleles per population. The allelic size pattern differed among the populations. In RJF, alleles were ranged from 274-465 bp followed by 255-417 bp in AS, 255-347 bp in RC and 255-270 bp in WLH. Expected heterozygosity was moderate to high and ranged from 0.5850 to 0.8086 in different populations. The observed heterozygosity was ranged from 0.4444 to 0.5000, while the PIC value was ranged from 0.5129 to 0.7816 in different populations. The estimates H_E, H_O and PIC were 0.8643, 0.4730 and 0.8523 respectively across all the populations.

4.1.1.11. Microsatellite LEI194

At Locus LEI194, number of alleles was ranged from 5 in RC to 8 in each RJF, WLH and AS. Across the population, a total of 17 alleles were observed within allelic size range from 118 bp to 184 bp. Expected heterozygosity was found high and ranged from 0.6960 to 0.8363 in different populations. The observed heterozygosity was found moderate to high and ranged from (0.2500 to 0.5882), while the PIC value was ranged from 0.6402 to 0.8156 in different populations. The estimates H_E, H_O and PIC were 0.8984, 0.4400 and 0.8898 respectively across all the populations.

4.1.1.12. Microsatellite LEI209

For LEI209 marker, 6-10 alleles were found in different populations, while across the population, total number of alleles was 18 and the allelic size range was from 136 bp to 196 bp. Expected heterozygosity was ranged from 0.6466 to 0.8500 in different populations. The observed heterozygosity was found moderate to high and ranged from 0.2778 to 0.6500, while the PIC value was ranged from 0.6033 to 0.8331 in different populations. The estimates H_E, H_O and PIC were 0.9038, 0.4474 and 0.8958 respectively across all the populations.

4.1.1.13. Microsatellite LEI212

For LEI212 marker, the highest number of 43 alleles was found in all populations. The number of 23 alleles was observed in RJF followed by 15 alleles in AS, 11 alleles in WLH and 5 alleles in RC populations. The allelic size range was from 294 bp to 492 bp. Expected heterozygosity was ranged from 0.5756 to 0.9404 in

different populations. The observed heterozygosity was found moderate to high and ranged from 0.3529 to 0.6842, while the PIC values was ranged from 0.5031 to 0.9374 in different populations. The H_E and PIC value at this locus were found maximum (0.9450 and 0.9426 respectively) across the populations and also within RJF population (0.9404 and 0.9374 respectively) as compare to these estimates on other loci.

4.1.1.14. Microsatellite LEI214

For LEI214 marker, 4-13 alleles were found in different populations, while across the population, total number of alleles was 24 and the allelic size range was from 134 bp to 332 bp. Expected heterozygosity was ranged from 0.2948 to 0.8255 in different populations. The observed heterozygosity was found low and ranged from 0.0526 to 0.3684, while the PIC value was ranged from 0.2797 to 0.8100 in different populations. The estimates H_E, H_O and PIC were 0.8994, 0.2329 and 0.8920 respectively across all the populations. RJF showed higher values of H_E, H_O and PIC as compare to domestic chickens.

4.1.1.15. Microsatellite LEI217

For LEI217 marker, 6-21 alleles were found in different populations, while across the population, total number of alleles was 29 and the allelic size range was from 148 bp to 300 bp. Expected heterozygosity was found high and ranged from (0.7400 to 0.9294) in different populations. The observed heterozygosity was ranged from 0.5000 to 0.8947, while the PIC value was found high and ranged from 0.6979 to 0.9252 in different populations. The estimates H_E, H_O and PIC were 0.9312, 0.6800 and 0.9271 respectively across all the populations. RJF showed higher values of H_E, H_O and PIC as compare to domestic chickens at this locus.

4.1.1.16. Microsatellite LEI221

For LEI221 marker, number of alleles were ranged from 2 in RC to 16 in RJF, however across the populations total number of alleles were 21. The allelic size range was from 120 bp to 244 bp. Expected heterozygosity was moderate to high and ranged from 0.4753 to 0.8712 in different populations. The observed heterozygosity was zero (in RC) to 0.6842 (in RJF), while the PIC value was found low to high and ranged from 0.3623 to 0.8604 in different populations. The estimates H_E, H_O and PIC were 0.8647, 0.3889 and 0.8516 respectively across all the populations. At locus

LEI221, RJF showed higher values of H_E, H_O and PIC as compare to domestic chickens.

4.1.1.17. Microsatellite LEI228

For LEI228 marker, 5-11 alleles were found in different populations, while across the population; total number of alleles was 15 and the allelic size range was from 190 bp to 266 bp. Expected heterozygosity was found high and ranged from 0.6275 to 0.7941 in different populations. The observed heterozygosity was ranged from 0.2353 to 0.4500, while the PIC value was found moderate to high and ranged from 0.5541 to 0.7652 in different populations. The estimates H_E, H_O and PIC were 0.8677, 0.3649 and 0.8553 respectively across all the populations.

4.1.1.18. Microsatellite LEI229

At locus LEI229, number of alleles were ranged from 3 in AS to 10 in RJF, however across the populations total number of alleles were 13. The allelic size range was from 193 bp to 355 bp. Expected heterozygosity was found low to high and ranged from 0.3642 to 0.8663 in different populations. The observed heterozygosity was ranged from 0.1111 to 0.4000, while the PIC value was ranged from 0.3267 to 0.8531 in different populations. The estimates H_E, H_O and PIC were 0.8770, 0.2133 and 0.8650 respectively across all the populations. At this locus, AS show lower values of H_E, H_O and PIC as compare to RJF and rest of domestic chicken breeds. The H_O, within AS population at this locus was found minimum (0.1111) as compare to H_O estimated in AS on other loci.

4.1.1.19. Microsatellite LEI232

At LEI 232 locus, 6-13 alleles were found in different populations, while across the population, total numbers of alleles were 22 and the allelic size range was from 178 bp to 290 bp. The expected heterozygosity was observed high and ranged from 0.7299 to 0.9028, while the observed heterozygosity was variable and ranged from 0.1667 to 0.5556 in different populations. The value of PIC was ranged from 0.6978 to 0.8947 in different populations. At this locus, PIC value within RC population was found maximum (0.8717) as compare to PIC value estimated in RC on other loci. The estimates H_E, H_O and PIC were 0.9127, 0.3472 and 0.9064 respectively across all the populations.

4.1.1.20. Microsatellite LEI234

At LEI234 locus, almost equal number of alleles were found in each population as 9 alleles in RC followed by 8-8 alleles in each RJF, WL and AS populations. Across all the populations, total numbers of alleles were 20 and the allelic size range was from 216 bp to 388 bp. The expected heterozygosity was observed high and ranged from 0.7188 to 0.8364, while the observed heterozygosity was high and ranged from 0.6111 to 0.9000 in different populations. The value of PIC was ranged from 0.6765 to 0.8169 in different populations. The estimates H_E, H_O and PIC were 0.9237, 0.6842 and 0.9186 respectively across all the populations. The H_O value at this locus was found maximum (0.6842) across all the populations as well as the H_O within RJF population was also maximum at this locus as compared to other chicken breeds.

4.1.1.21. Microsatellite LEI237

For LEI237 marker, 8-15 alleles were found in different populations, while across the population, total number of alleles was 28 and the allelic size range was from 211 bp to 391 bp. Expected heterozygosity was found high and ranged from 0.7630 to 0.9155 in different populations. The observed heterozygosity was ranged from 0.3684 to 0.8333, while the PIC value was found high and ranged from 0.7318 to 0.9094 in different populations. The estimates H_E, H_O and PIC were 0.9431, 0.6164 and 0.9403 respectively across all the populations. At this locus, H_O within AS population was found maximum (0.8333) as compare to Ho estimated in AS on other loci.

4.1.1.22. Microsatellite LEI243

For LEI243 marker, 6-13 alleles were found in different populations, while across the population, total number of alleles was 24 and the allelic size range was from 170 bp to 364 bp. Expected heterozygosity was found high and ranged from 0.6188 to 0.8925 in different populations. The observed heterozygosity was found low to high and ranged from 0.0500 to 0.6111, while the PIC value was found moderate to high and ranged from 0.5837 to 0.8833 in different populations. The estimates H_E, H_O and PIC were 0.9194, 0.3684 and 0.9140 respectively across all the populations.

4.1.1.23. Microsatellite LEI246

At locus LEI246, 7-10 alleles were found in different populations, while across the population, total number of alleles was 17 and the allelic size range was

from 199 bp to 271 bp. Expected heterozygosity was ranged from 0.6605 to 0.8767 in different populations. The observed heterozygosity was ranged from 0.0526 to 0.7222, while the PIC value was ranged from 0.6244 to 0.8640 in different populations. The estimates H_E, H_O and PIC were 0.9043, 0.3784 and 0.8968 respectively across all the populations. At this locus, observed heterozygosity within RJF population was found minimum (0.0526) as compare to Ho estimated in RJF on other loci.

4.1.1.24. Microsatellite LEI248

For LEI248 marker, 5-8 alleles were found in different populations, while across the population, total number of alleles was 11 and the allelic size range was from 222 bp to 262 bp. Expected heterozygosity was found high and ranged from 0.6950 to 0.8040 in different populations. The observed heterozygosity was ranged from 0.0000 to 0.6667, while the PIC value was found high and ranged from 0.6428 to 0.7766 in different populations. The estimates H_E, H_O and PIC were 0.7941, 0.3919 and 0.7667 respectively across all the populations.

4.1.1.25. Microsatellite MCW111

For MCW111 marker, comparatively fewer alleles were found. For MCW111 marker, 3-4 alleles were found in different populations, while across the populations, total numbers of alleles were 5 in the range of 98 bp to 106 bp. Expected heterozygosity ranged from 0.5150 to 0.6235 in different populations, while the observed heterozygosity were moderate to high and ranged from 0.3889 to 0.6111. PIC value was from 0.4244 to 0.5443 in different populations. The estimates H_E, H_O and PIC were 0.7285, 0.4730 and 0.6814 respectively across the populations. The H_E and PIC value (0.7285 and 0.6814 respectively) at this locus were found minimum across the populations and also within RJF population (0.6065 and 0.5313 respectively) as compare to these estimates on other loci.

4.1.1.26. Across all microsatellite loci

A total of 475 alleles with an average of 19 alleles per locus were produced with 25 microsatellite markers. The highest number of 248 alleles with an average of 9.92 alleles per locus were observed in RJF followed by 212 alleles with an average of 8.48 alleles per locus in AS, 177 alleles with an average of 7.08 alleles per locus in each of WLH and RC populations. The number of alleles at each locus ranged from 5 (MCW111) to 43 (LEI212). The RJF population showed higher values of means of

H_E, H_O and PIC (0.7975, 0.4565 and 0.7716) as compared to the three domestic chicken populations.

4.1.2. Population specific alleles

Allelic frequencies of different alleles at various microsatellite loci in different populations are presented in **Table 4.3 to 4.27**. Perusal of these tables revealed a number of population-specific alleles in different populations at every microsatellite loci. Population-specific alleles with their respective frequencies are presented in **Table 4.28**. Overall, a total of 242 population-specific unique alleles were found at 25 microsatellite loci. The number of population specific allele at one locus ranged from 3 (at LEI248 locus) to 32 alleles (at LEI212 locus). Of these, 103 alleles were specific to RJF population only followed by 56 alleles specific to AS, 44 alleles specific to WLH and 39 alleles specific to RC.

At ADL34 locus, two alleles were found specific to WLH (125 bp and 151bp) followed by 2-2 allele in each AS and RC. No specific allele was observed for RJF at this locus. At ADL120 locus, 10 population specific alleles were observed. Out of 10, 3 were specific to RJF and 7 were specific to AS. The 150 bp allele (AS specific) was present in high frequency (0.41). At locus ADL158, 3 alleles (172 bp, 174 bp and 176 bp) were found specific to RJF and one allele (208 bp) was specific to RC.

At locus ADL209, total 7 alleles were found population-specific. Out of 7, 4 alleles (132 bp, 158 bp, 166 bp and 178 bp) were found specific to RC followed by 2 alleles (138 bp and 140 bp) in AS and one allele (160 bp) in RJF. At locus ADL254, total 9 population-specific alleles were found. 8 alleles were specific to AS only with a size range from 152 bp to 180 bp and only one allele was specific to RC. No specific band was found in RJF and WLH. At locus ADL265, 3-3 alleles were found specific to RJF and WLH each and one allele was specific to AS. No specific allele was found in RC.

At locus ADL 270, total 6 population-specific alleles were found. Out of 6, 2 alleles were specific to RJF (90 bp and 100 bp) and 2 alleles in each AS (116 bp and 126 bp) and RC (130 bp and 132 bp). No specific allele was found in WLH. At locus ADL 327, total 9 population-specific alleles were present across all populations. Out of 9 alleles, 5 alleles were present in RC followed by 3 alleles in AS and 1 allele in RJF. One allele (110 bp) in RC was present with a high frequency (0.35) as compare to other alleles. At locus ADL 331, population-specific alleles were present in all four

populations. Total 6 specific alleles were found; in which 2-2 alleles were specific to AS and RC populations and 1-1 allele were specific to RJF and WLH populations respectively.

At locus LEI194, total 9 specific alleles were found. 4 alleles were specific to RJF followed by 3 alleles to WLH and 2 alleles to AS. No specific allele was found for RC population. At locus LEI209, two alleles were specific to RJF (136 bp and 162 bp) with same allelic frequencies 0.0250 for each. One allele (142 bp) was specific to WLH and 4 alleles were found specific to AS. At locus LEI212, locus LEI214 and locus LEI217 most of the specific alleles were RJF-specific alleles. Locus 212 was found very informative and highly polymorphic between RJF and other three domestic chicken populations. This locus had specific alleles for all four populations. Total 32 specific alleles were found at this locus that number was maximum present at any microsatellite locus in this study. Out of 32, 18 alleles were specific to RJF followed by 6 - 6 alleles specific to WLH and AS each and 2 alleles specific to RC. Out of the two alleles, one allele (412 bp) that was specific to RC, was present in high frequency (0.33).

At locus LEI214, total 18 specific alleles were found at this locus. Out of 18, 11 alleles were specific to RJF followed by 6 alleles specific to RC. One specific allele (140 bp) was also present in AS with a very high allele frequency (0.8333). At locus LEI217, total 16 specific alleles were found at this locus. Out of 16, 11 alleles were specific to RJF followed by 2 alleles specific to each WLH and AS and 1 allele was specific to RC. One (148 bp) between the two alleles specific to WLH was present with a high allele frequency (0.37). At locus LEI221, 7 allele were specific to RJF followed by 4 specific to WLH and one specific to AS population.

At locus LEI228, total 6 alleles were found specific to all four populations. 3 allele were specific to RJF, 2 alleles to WLH and one allele to AS. At locus LEI229, total 7 alleles were found specific to all four populations. 5 allele were specific to RJF and 1 allele to each AS and RC. At Locus LEI232, total 10 specific alleles were found at this locus. Out of 10, 4 alleles were specific to RJF followed by 5 alleles specific to WLH and one allele specific to RC. No allele was found specific to AS population. At locus LEI234, population-specific alleles were present in all four populations. 5 alleles were specific to RC followed by 4 alleles specific to RJF and 1 allele specific to WLH and AS each. At locus LEI237, total 13 specific alleles were found. Out of

13, 8 alleles were specific to WLH followed by 4 alleles to RJF and 1 allele specific to AS. No specific allele was found for RC population.

At locus LEI243, population-specific alleles were present in all four populations. Total 14 specific alleles were found. Out of 14, 8 alleles were specific to RJF followed by 4 alleles specific to RC and 1 allele specific to AS. One WLH specific allele (186 bp) with high allele frequency (0.575) was also found. At locus LEI246, total 6 specific alleles were present. 4 were specific to WLH and one allele specific to RJF and RC each. At Locus LEI248, 3 population specific alleles were found. All three alleles (222 bp, 258 bp and 262 bp) were specific to AS population only. No allele was found specific in other three populations.

Genotyping of two markers LEI192 and MCW111 was done on capillary-based electrophoresis system (3130xlGenetic Analyzer). On capillary-based systems the accuracy of allelic sizing is very high and accurate relative to gel-based electrophoresis systems. So, allele sizing at these two loci was very accurate. At Locus LEI192, total 12 population specific alleles were present. Out of 12, 5 alleles were specific to RJF followed by 5 alleles specific to AS and 1 allele specific to WLH and RC each. Allelic frequencies of RJF specific alleles were 274 bp (0.2778), 370 bp (0.2222), 393 bp (0.1111), 401 bp (0.1667) and 465 bp (0.1111). At Locus MCW 111, minimum number of only 2 population-specific alleles 104 bp and 106 bp were found with high allelic frequencies 0.50 for each. Both the alleles were specific to RJF population. No allele was found specific in other three populations.

4.1.3. Genetic Differentiation and Gene Flow

Genetic differentiation was examined by fixation indices F_{IS}, F_{IT} and F_{ST} for each locus across populations and the estimates have been presented in **Table 4.29**. The F_{IT} [inbreeding coefficient of an individual (I) relative to the total population (T)] value, for the 25 microsatellite loci varied from 0.2649 (LEI234) to 0.8024 (ADL120) with a mean of 0.5068. The F_{IS} [inbreeding coefficient of an individual (I) relative to the sub-population (S)] value, for the 25 microsatellite loci varied from 0.1449 (LEI234) to 0.6615 (LEI229) with a mean of 0.4047. The F_{ST} [effect of sub-populations (S) compared to the total population (T)] value, across all microsatellite loci varied from 0.0580 (ADL254) to 0.5530 (ADL120) with a mean of 0.1716.

Genetic differentiation, examined by fixation indices F_{IS}, F_{IT} and F_{ST} between the populations, across all the microsatellite loci are presented in **Table 4.30**. The F_{IT}

values ranged from 0.3699 between AS & RC to 0.4380 between RJF & WLH. Similarly, the F_{IT} estimates ranged from 0.4504 between AS & RC to 0.4963 between RJF and WLH. The F_{ST} [effect of sub-populations (S) compared to the total population (T)] value was found minimum (0.1027) between RJF and AS while maximum (0.1443) between WLH and RC. The F_{ST} value between RJF and domestic chicken populations were lower than the F_{ST} value among domestic chicken populations.

Gene flow (Nm) for each locus across populations is presented in **Table 4.29**. The Nm values ranged from 0.2021 (ADL120) to 4.0612 (ADL254) with a mean of 1.2070. Gene flow between the populations across all the microsatellite loci is shown in **Table 4.30**. Gene flow between RJF and the domestic chicken breeds i.e. WLH, AS and RC was found to be 2.1593, 2.1840 and 1.7921 respectively. Between domestic chicken breeds, gene flow was observed lower in comparison to the gene flow estimated between wild RJF and domestic chicken breeds. Between domestic chicken populations gene flow was observed 1.7057 (between AS and RC) followed by 1.6984 (between WLH and AS) and 1.4822 (between WLH and RC).

4.1.4. Genetic relatedness and phylogenetic analysis

Using the allelic frequencies, shared allele based genetic distances between the populations was estimated using PowerMarker software and has been presented in **Table 4.31**. Genetic distance between RJF and domestic chicken populations namely as WLH, AS and RC was observed 0.7562, 0.7689 and 0.8093 respectively. Within domestic chicken breeds, genetic distances was ranged from 0.7302 between AS and RC to 0.7637 between WLH and RC.

To understand phylogenetic relationships an UPGMA dendrogram was constructed from a data matrix of shared allele frequencies between RJF and domestic chickens **(Fig 4.26- A)**. At about 60% genetic similarity, all four populations were divided in to two main clusters. The Wild RJF and WLH were placed in cluster-I and AS and RC were placed in cluster-II. Again at about 65% genetic similarity, both the mains clusters divided in to two sub clusters of each. At this level, all four populations were formed a separate sub-group. However, clustering of RJF with WLH indicated relatively close genetic relationship between the two. Similarly, clustering of RC with AS showed close genetic relationship between the two. The UPGMA dendrogram

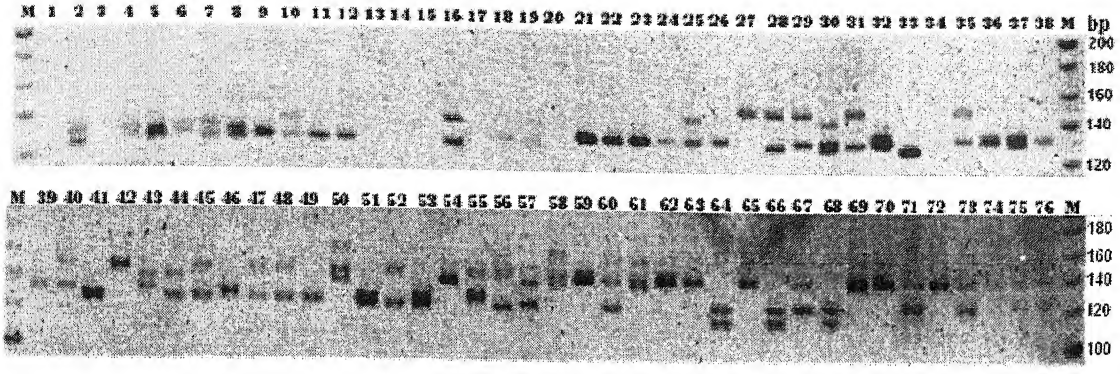


Fig 4.1. Microsatellite allelic profile of different Chicken populations generated with marker **ADL034**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

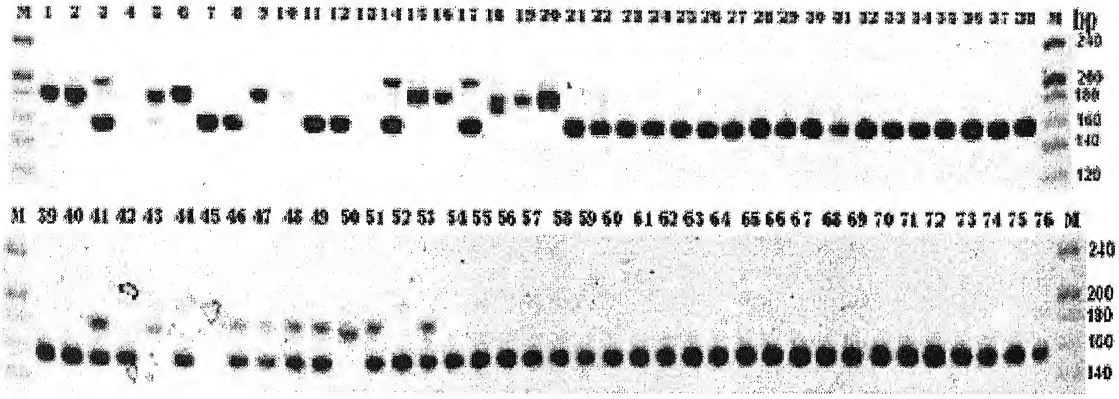


Fig 4.2. Microsatellite allelic profile of different Chicken populations generated with marker **ADL120**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

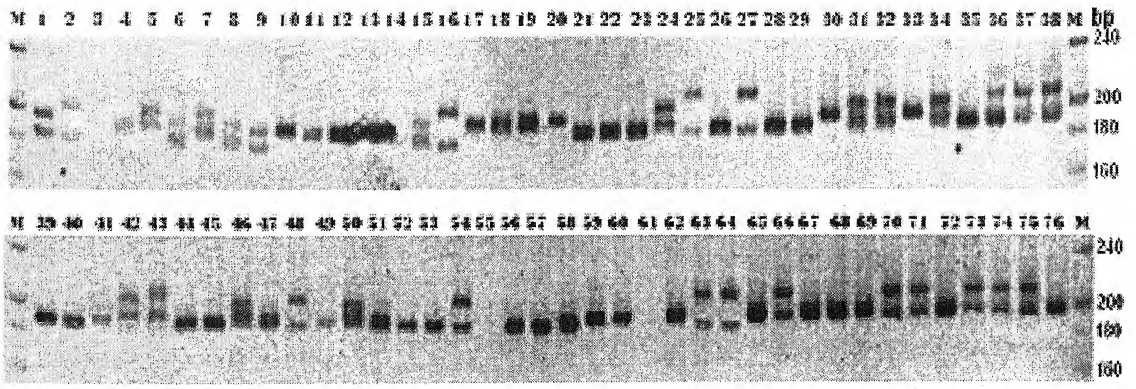


Fig 4.3. Microsatellite allelic profile of different Chicken populations generated with marker **ADL158**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

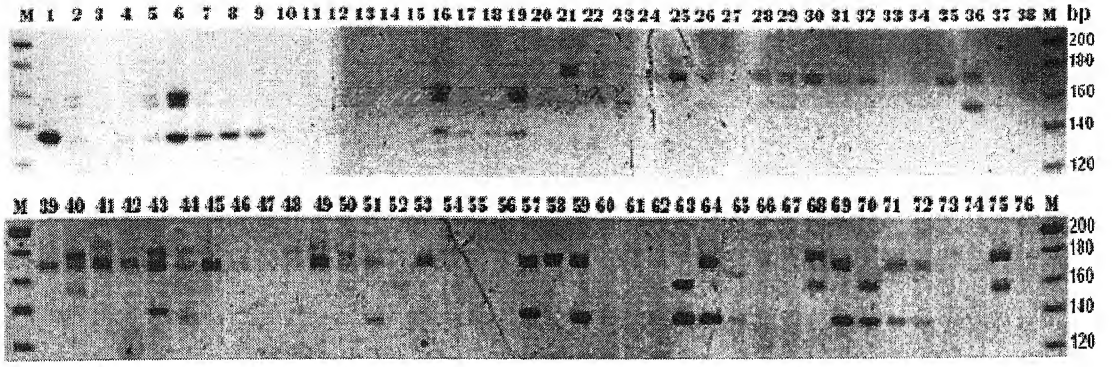


Fig 4.4. Microsatellite allelic profile of different Chicken populations generated with marker **ADL209**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

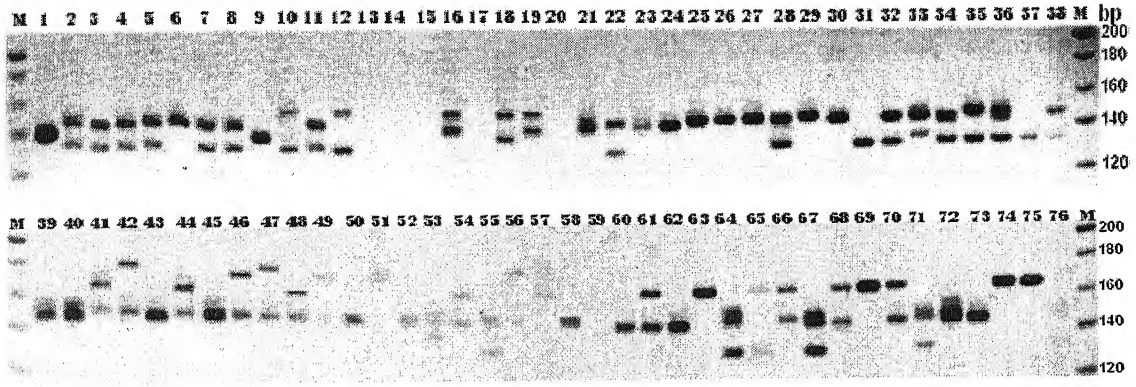


Fig 4.5. Microsatellite allelic profile of different Chicken populations generated with marker **ADL254**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

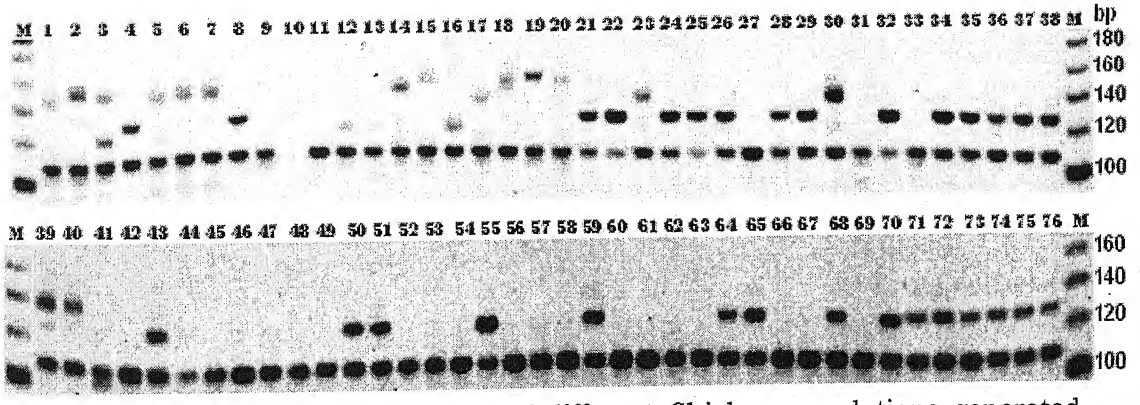


Fig 4.6. Microsatellite allelic profile of different Chicken populations generated with marker **ADL265**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

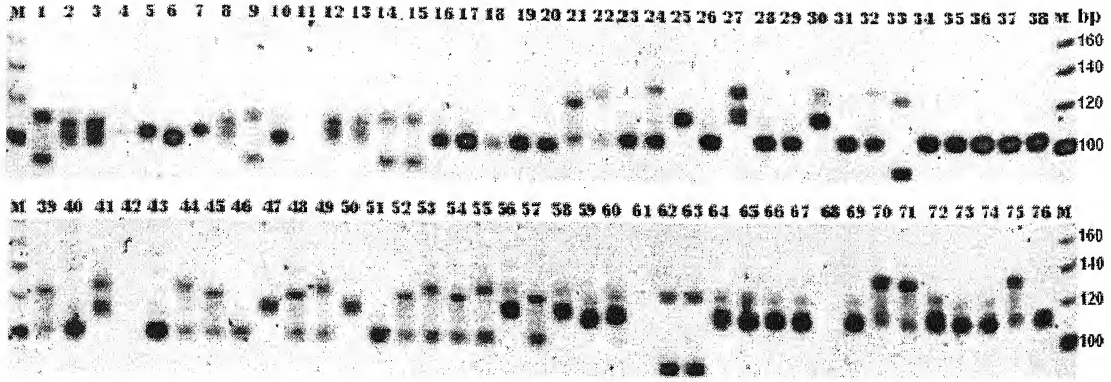


Fig 4.7. Microsatellite allelic profile of different Chicken populations generated with marker **ADL270**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

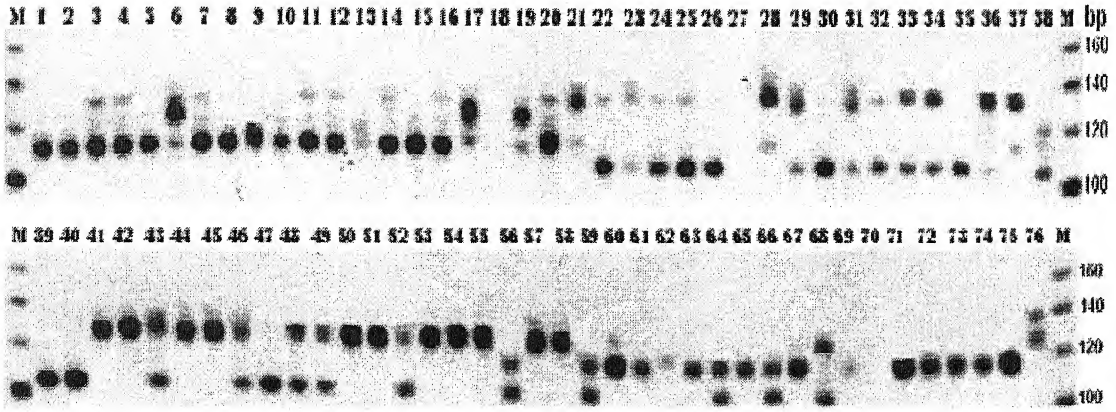


Fig 4.8. Microsatellite allelic profile of different Chicken populations generated with marker **ADL327**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

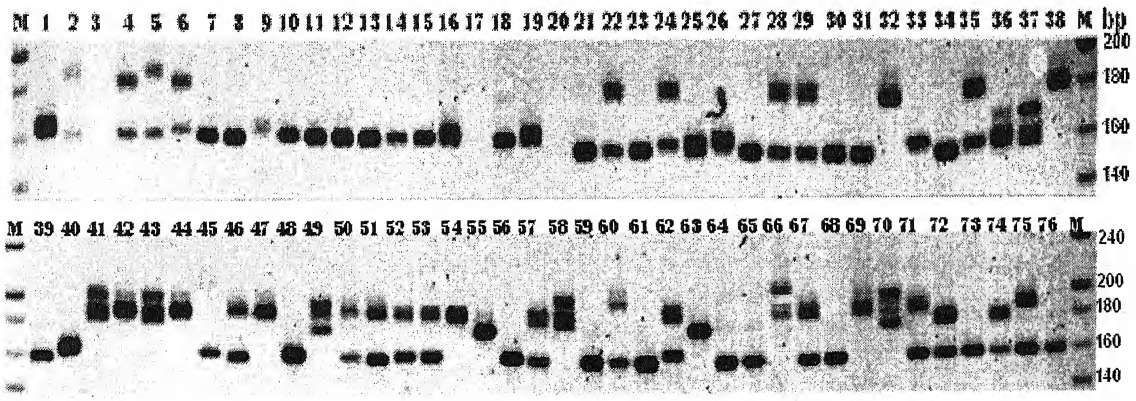


Fig 4.9. Microsatellite allelic profile of different Chicken populations generated with marker **ADL331**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

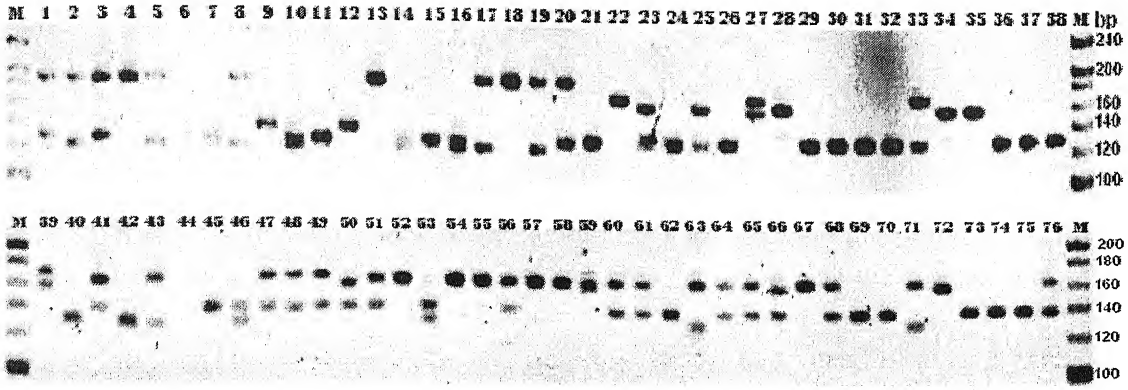


Fig 4.10. Microsatellite allelic profile of different Chicken populations generated with marker **LEI194**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

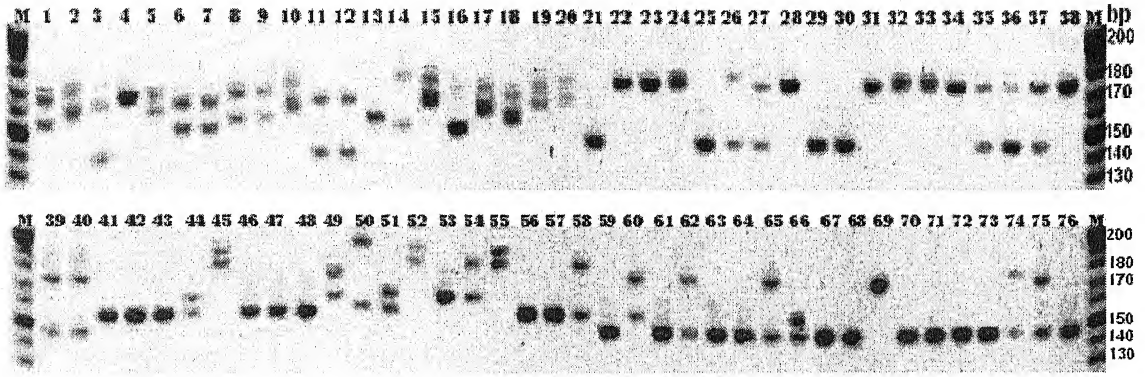


Fig 4.11. Microsatellite allelic profile of different Chicken populations generated with marker **LEI209**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

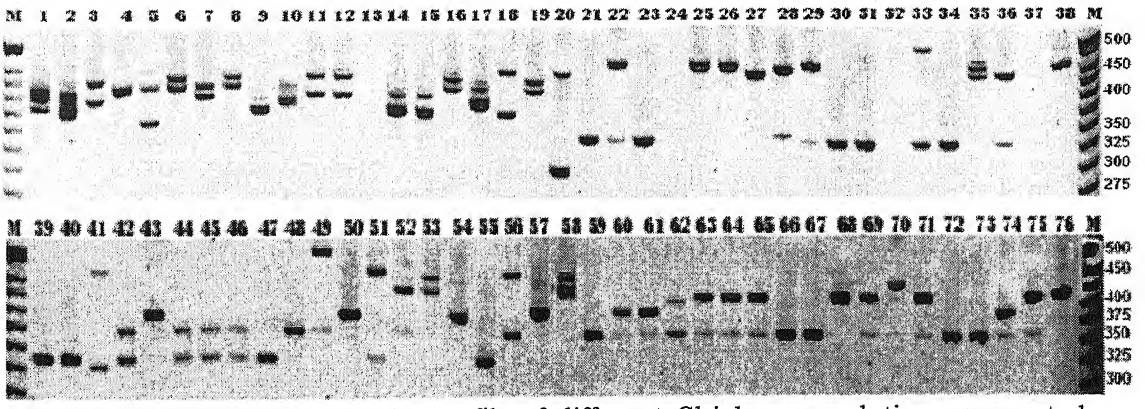


Fig 4.12. Microsatellite allelic profile of different Chicken populations generated with marker **LEI212**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

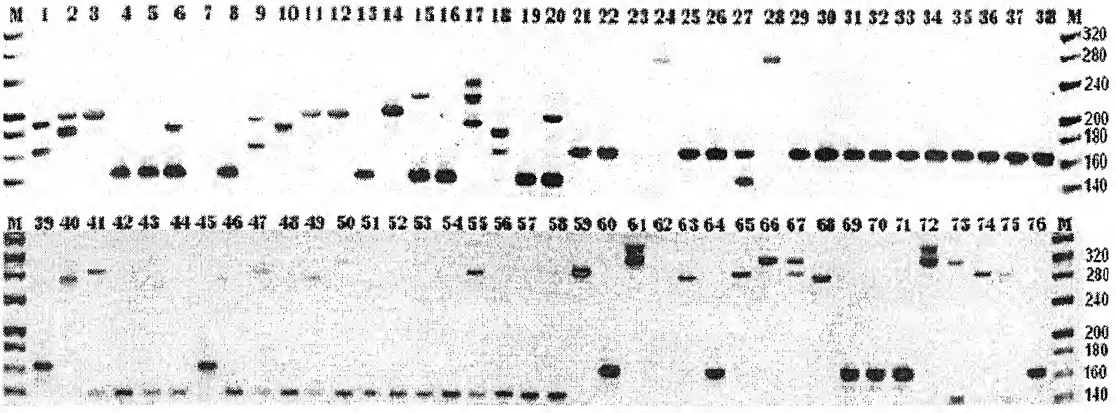


Fig 4.13. Microsatellite allelic profile of different Chicken populations generated with marker **LEI214**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

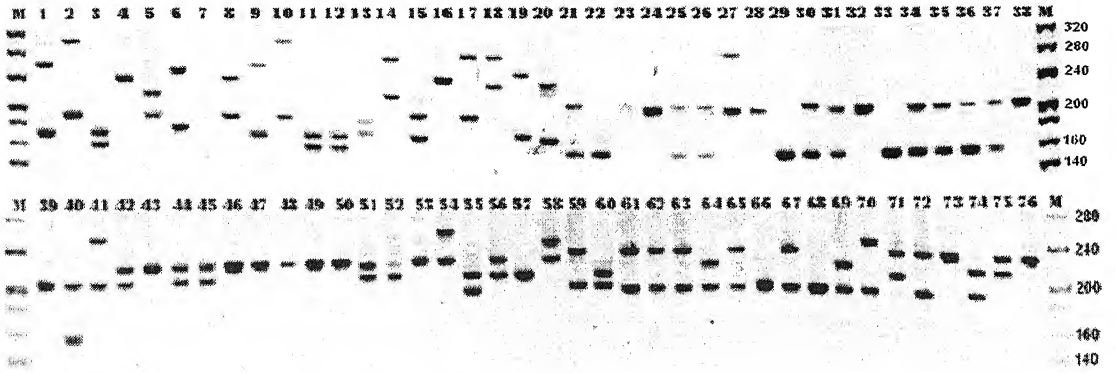


Fig 4.14. Microsatellite allelic profile of different Chicken populations generated with marker **LEI217**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

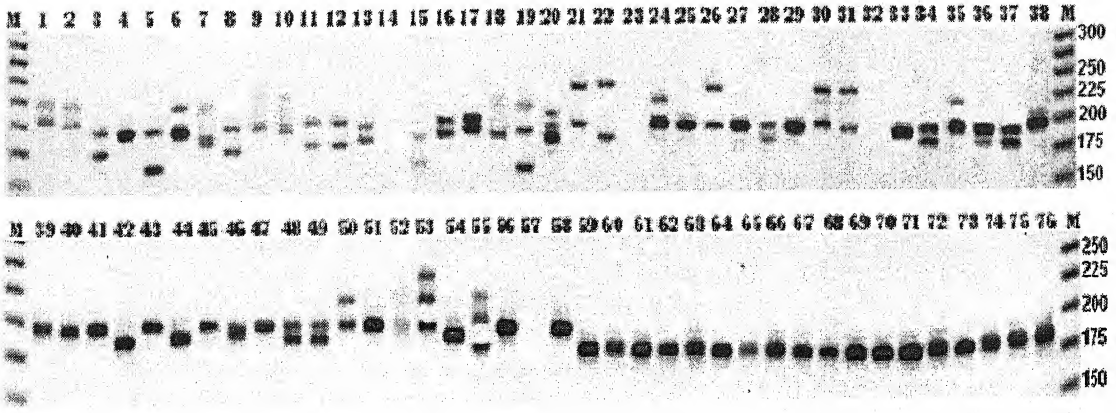


Fig 4.15. Microsatellite allelic profile of different Chicken populations generated with marker **LEI221**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

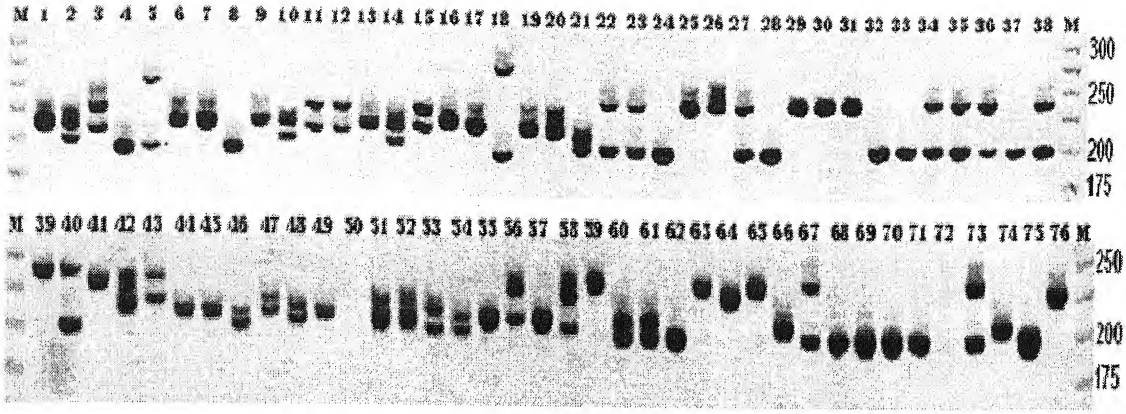


Fig 4.16. Microsatellite allelic profile of different Chicken populations generated with marker **LEI228**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

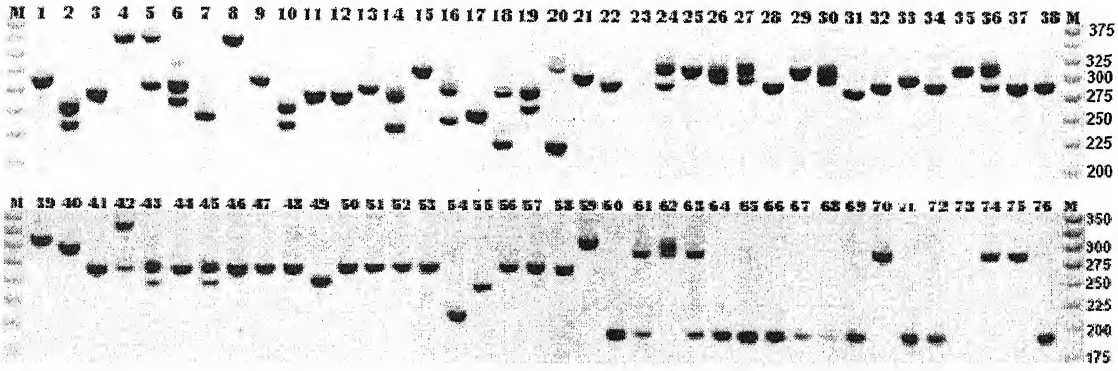


Fig 4.17. Microsatellite allelic profile of different Chicken populations generated with marker **LEI229**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

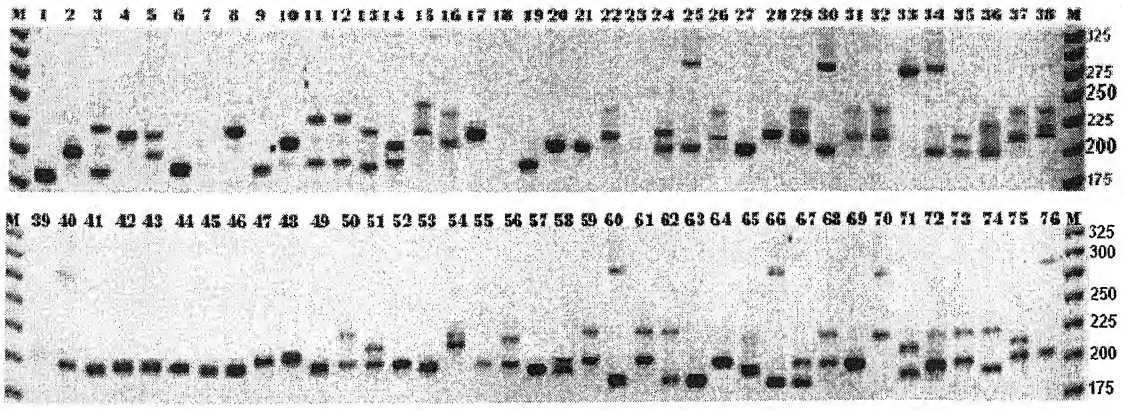


Fig 4.18. Microsatellite allelic profile of different Chicken populations generated with marker **LEI232**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

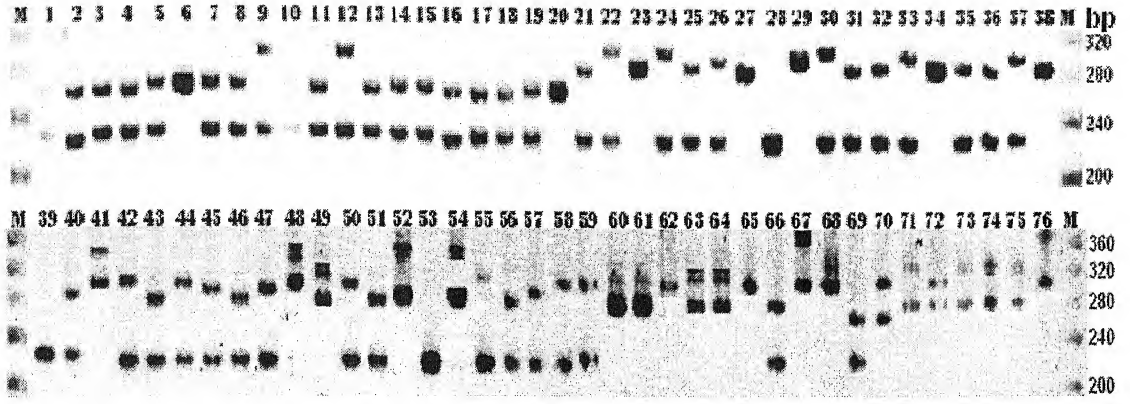


Fig 4.19. Microsatellite allelic profile of different Chicken populations generated with marker **LEI234**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

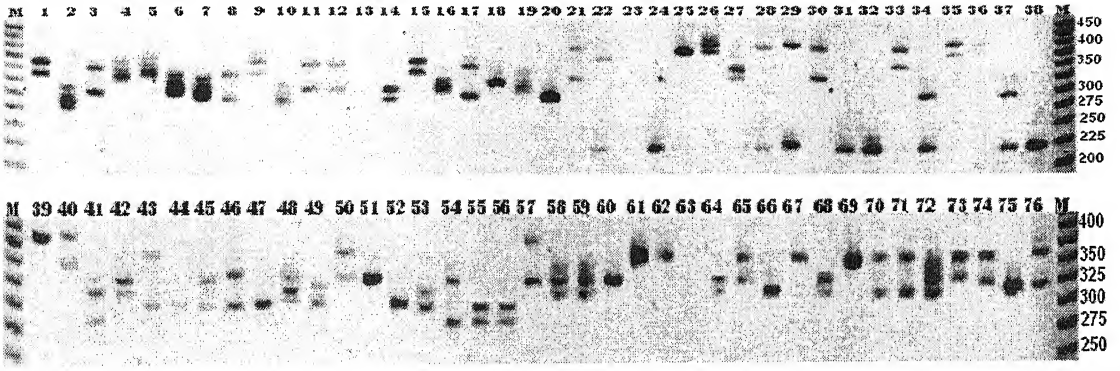


Fig 4.20. Microsatellite allelic profile of different Chicken populations generated with marker **LEI237**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

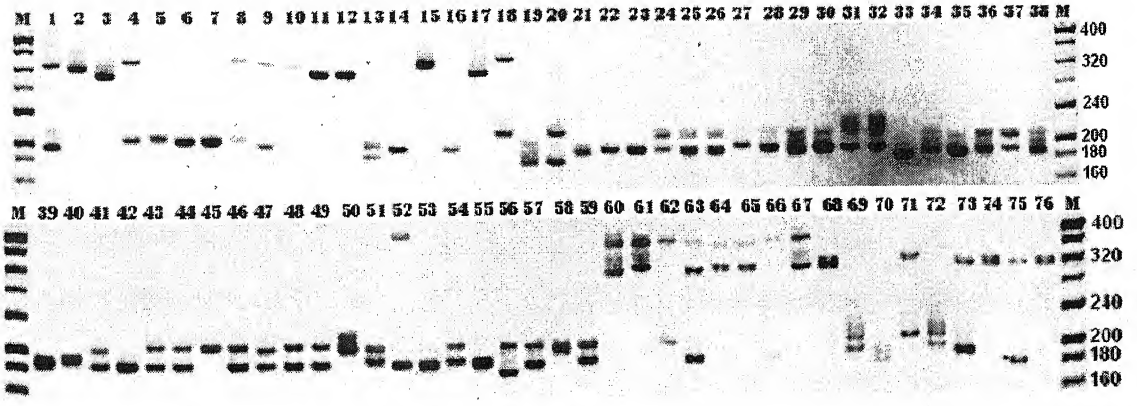


Fig 4.21. Microsatellite allelic profile of different Chicken populations generated with marker **LEI243**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

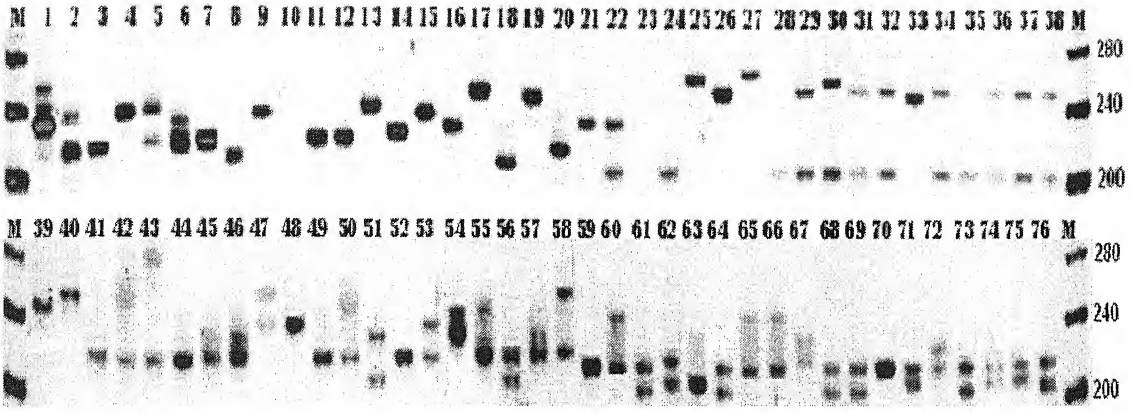


Fig 4.22. Microsatellite allelic profile of different Chicken populations generated with marker **LEI246**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

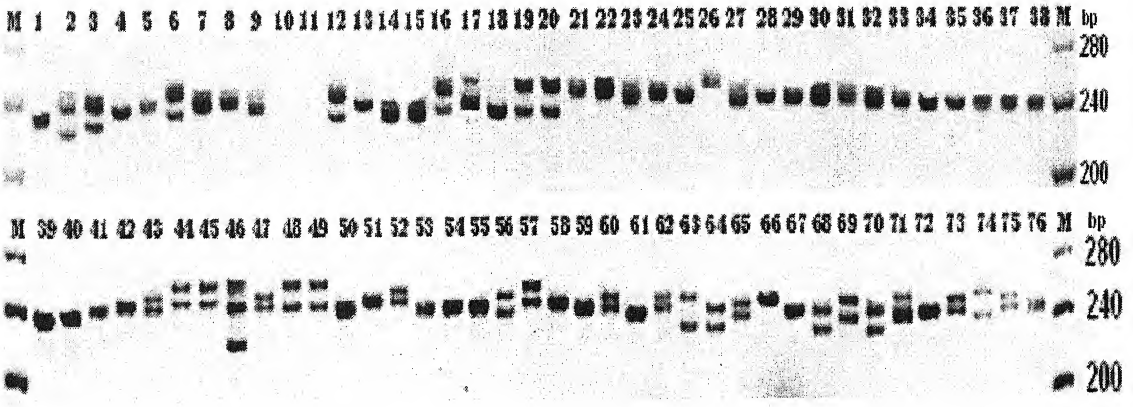


Fig 4.23. Microsatellite allelic profile of different Chicken populations generated with marker **LEI248**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

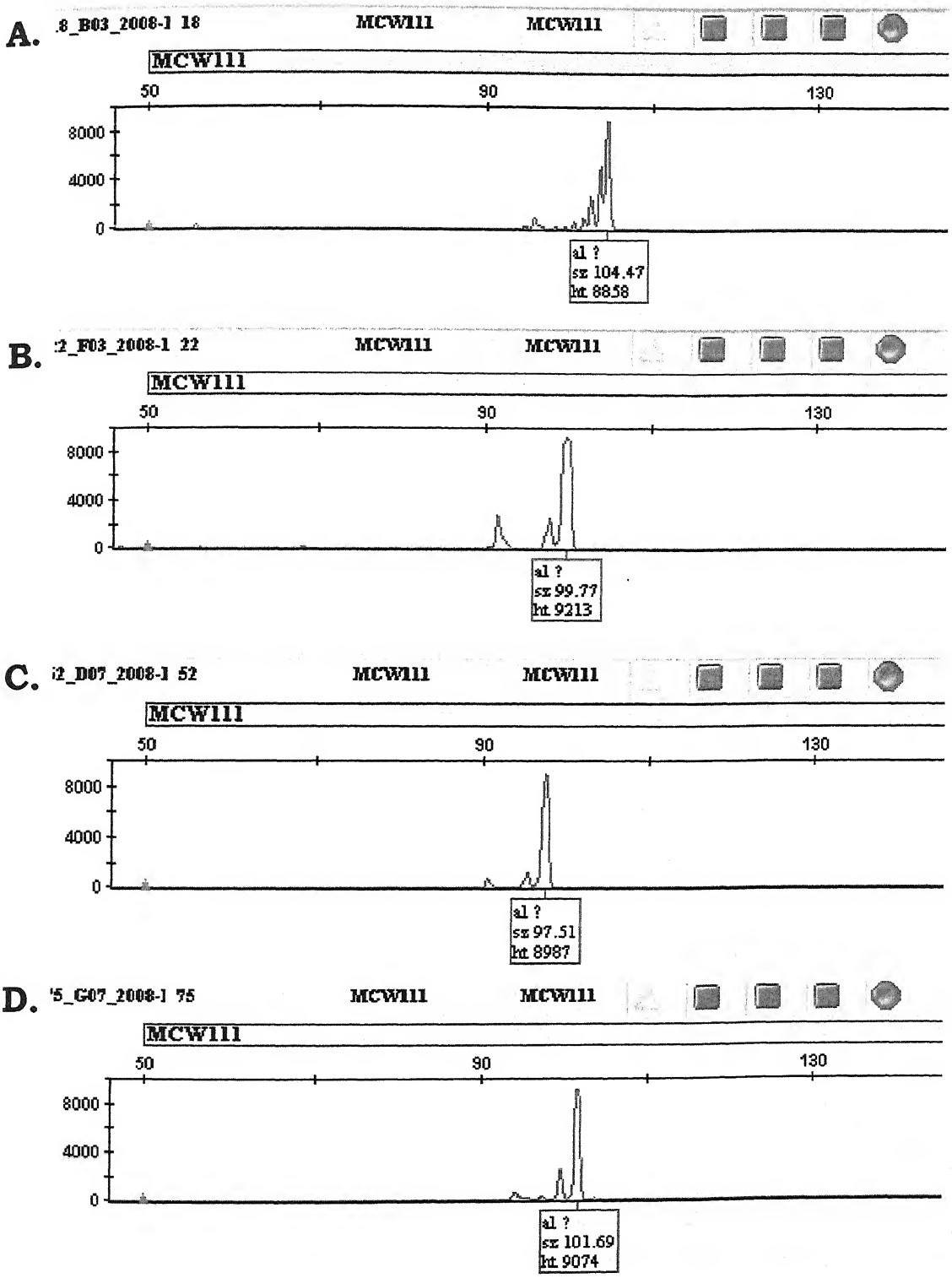


Fig 4.24. Microsatellite allelic profile of 4 individuals generated with marker **MCW111**. A. individual 18 of RJF, B. individual 22 of White Leghorn, individual 52 of Aseel and individual 75 of Red Cornish

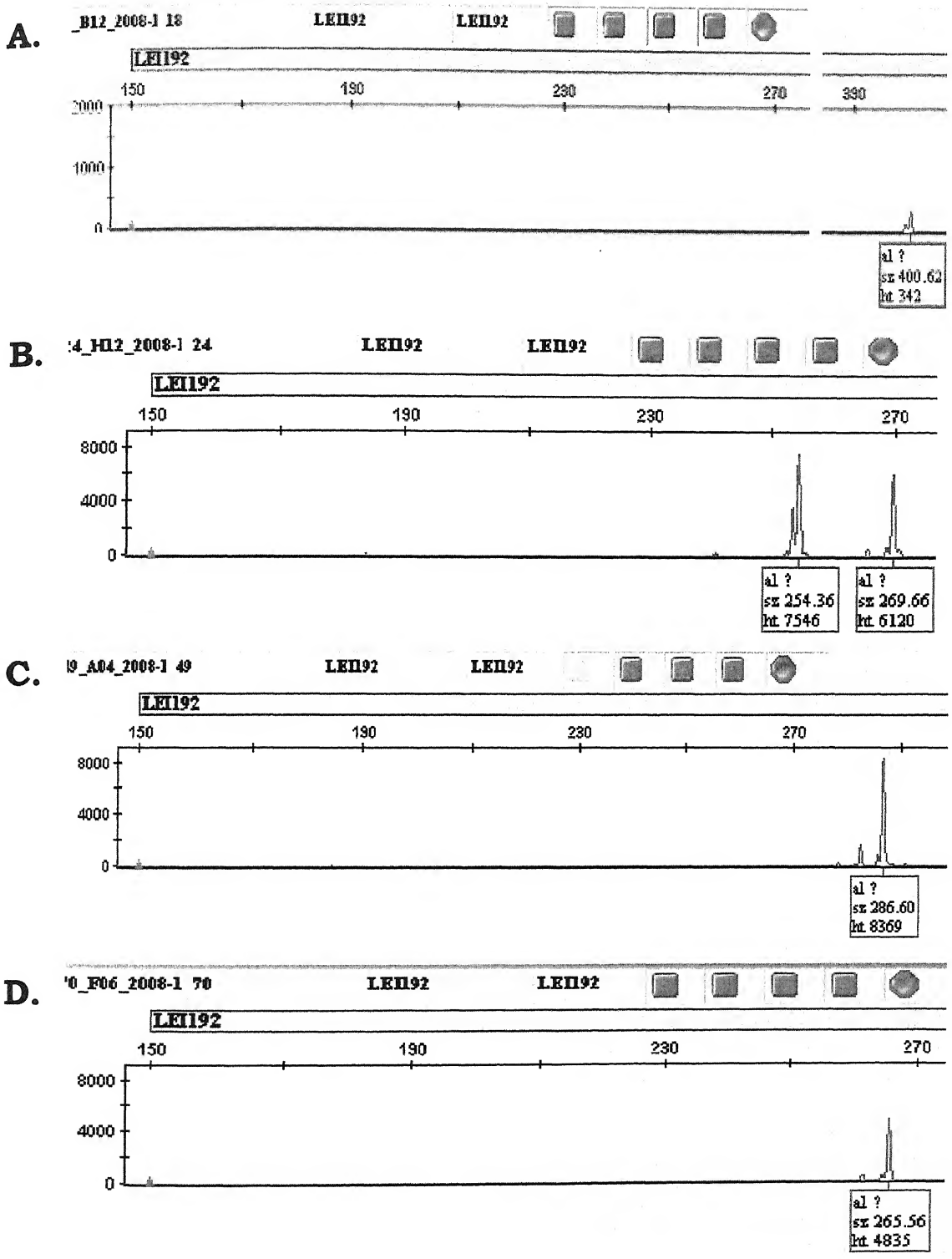


Fig 4.25. Microsatellite allelic profile of 4 individuals generated with marker **LEI192**. A. individual 18 of RJF, B. individual 24 of White Leghorn, individual 49 of Aseel and individual 70 of Red Cornish

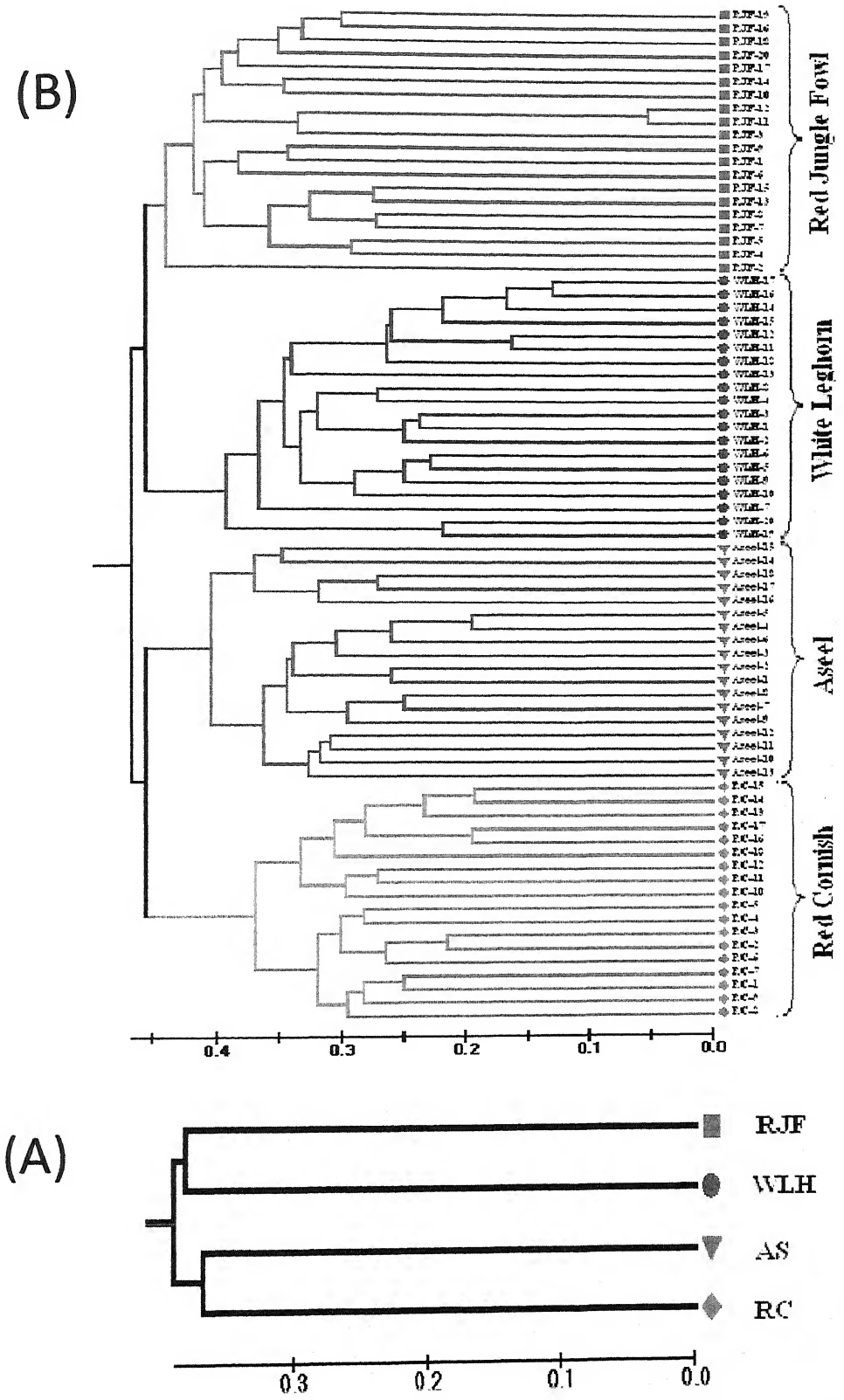


Fig. 4.26. UPGMA dendrogram constructed from a data matrix of shared allele frequencies showing genetic relationships among **(A).** Red Jungle Fowl (RJF) and domestic chickens namely White Leghorn (WLH), Aseel (AS) and Red Cornish (RC) and **(B).** 76 individuals of wild and domestic chickens using Microsatellite markers

Table 4.1. Number of alleles and allelic size range in RJF and different chicken breeds at different microsatellite loci

Micro-satellite loci	Red Jungle Fowl		White Leghorn		Aseel		Red Cornish		Overall population	
	Number of alleles	Size range bp	Number of alleles	Size range bp	Number of alleles	Size range bp	Number of alleles	Size range bp	Number of alleles	Size range bp
ADL034	8	127-143	8	125-151	13	121-159	6	113-153	18	113-159
ADL120	4	156-192	1	**156**	8	146-174	1	**152**	12	146-192
ADL158	10	172-198	9	180-204	7	182-204	6	184-208	13	172-208
ADL209	4	134-160	5	154-176	10	134-176	11	132-178	15	132-178
ADL254	8	130-150	10	128-150	**18**	126-180	11	126-164	24	126-180
ADL265	7	106-134	8	104-144	5	100-124	4	102-124	15	100-144
ADL270	6	090-110	8	086-128	9	100-128	7	086-132	17	086-132
ADL327	6	112-132	7	104-134	7	102-130	8	098-134	17	098-134
ADL331	6	159-191	10	151-179	13	153-197	**13**	151-197	21	151-197
LEI192	6	274-465	3	255-270	8	255-417	4	255-347	16	255-465
LEI194	8	118-184	8	120-168	8	128-166	5	128-164	17	118-184
LEI209	10	136-176	6	142-180	10	152-196	7	140-176	18	136-196
LEI212	**23**	294-444	11	328-484	15	322-492	5	360-428	**43**	294-492
LEI214	13	144-224	5	144-280	4	140-284	8	134-332	24	134-332
LEI217	21	156-300	6	148-272	10	200-264	11	192-248	29	148-300
LEI221	16	120-224	11	180-244	6	176-212	2	172-176	21	120-244
LEI228	11	190-266	5	194-238	9	202-234	6	194-230	15	190-266
LEI229	10	220-355	4	283-310	3	211-265	4	193-310	13	193-355
LEI232	13	180-232	10	194-290	6	188-216	12	178-220	22	178-290
LEI234	8	224-304	8	224-304	8	216-304	9	220-388	20	216-388
LEI237	15	283-359	**12**	211-391	11	279-375	8	307-355	28	211-391
LEI243	13	172-332	6	180-200	6	170-200	12	176-364	24	170-364
LEI246	10	211-259	8	203-271	7	203-255	7	199-239	17	199-271
LEI248	8	226-254	5	238-254	8	222-262	7	226-250	11	222-262
MCW111	4	100-106	3	098-102	3	098-102	3	098-102	5	098-106
Overall	**248**	**090-465**	**177**	**086-484**	**212**	**098-492**	**177**	**086-428**	**475**	**086-492**
Mean	**9.92**		**7.08**		**8.48**		**7.08**		**19**	

Table 4.2. Expected heterozygocity (H_E), observed heterozygocity (Ho) and polymorphic information content (PIC) measured in RJF and different chicken breeds at different microsatellite loci

Micro-satellite loci	Red Jungle Fowl			White Leghorn			Aseel			Red Cornish			Overall population		
	H_E	Ho	PIC	H_E	Ho	PIC	H_E	Ho	PIC	H_E	Ho	PIC	H_E	Ho	PIC
ADL034	0.7781	0.4615	0.7487	0.7175	0.3684	0.6893	0.8596	0.6111	0.8454	0.6194	0.5882	0.5752	0.9000	0.5075	0.8924
ADL120	0.6330	0.2105	0.5709	**0.0000**	**0.0000**	**0.0000**	0.7751	0.4118	0.7539	**0.0000**	**0.0000**	**0.0000**	0.7780	**0.1486**	0.7492
ADL158	0.8407	0.4737	0.8222	0.8375	0.4500	0.8202	0.8304	0.3529	0.8081	0.7647	0.4706	0.7332	0.8909	0.4384	0.8808
ADL209	0.6403	0.3571	0.5738	0.7128	0.3529	0.6719	0.8467	0.5333	0.8296	0.8472	**0.8333**	0.8322	0.8783	0.5000	0.8661
ADL254	0.8622	0.8000	0.8463	0.8438	0.4000	0.8253	**0.8935**	0.6667	**0.8855**	0.8789	0.5294	0.8666	0.9223	0.5857	0.9172
ADL265	0.6593	**0.1053**	0.6217	0.7825	**0.8000**	0.7493	0.7130	0.2222	0.6614	0.6898	0.6111	0.6381	0.8858	0.4400	0.8753
ADL270	0.7355	0.4737	0.7003	0.6350	0.2500	0.6115	0.8443	0.5882	0.8258	0.7129	0.3125	0.6734	0.8401	0.4028	0.8266
ADL327	0.6953	0.2105	0.6476	0.6274	0.7368	0.5648	0.5895	0.3333	0.5610	0.7145	0.2941	0.6700	0.8862	0.3973	0.8754
ADL331	**0.6055**	0.2353	0.5706	**0.8675**	0.3000	**0.8534**	0.8735	0.5556	0.8621	0.8426	0.4444	0.8264	0.9159	0.3836	0.9100
LEI192	0.8086	0.4444	0.7816	0.5850	0.5000	0.5129	0.7932	0.4444	0.7656	0.6991	0.5000	0.6435	0.8643	0.4730	0.8523
LEI194	0.8363	0.4500	0.8156	0.7038	0.2500	0.6696	0.7353	0.5882	0.6991	0.6960	0.5000	0.6402	0.8984	0.4400	0.8898
LEI209	0.8500	0.6500	0.8331	0.7475	0.3500	0.7046	0.8102	0.5000	0.7885	0.6466	0.2778	0.6033	0.9038	0.4474	0.8958
LEI212	**0.9404**	0.6842	**0.9374**	0.8478	0.3529	0.8304	0.8858	0.5556	0.8758	0.5756	0.6111	0.5031	**0.9450**	0.5556	**0.9426**
LEI214	0.8255	0.3684	0.8100	0.5942	0.0526	0.5451	**0.2948**	0.2222	**0.2797**	0.8097	0.2941	0.7899	0.8994	0.2329	0.8920
LEI217	0.9294	0.8947	0.9252	0.7400	0.5500	0.6979	0.8040	0.5000	0.7837	0.8704	0.7778	0.8573	0.9312	0.6800	0.9271
LEI221	0.8712	0.6842	0.8604	0.8040	0.5556	0.7805	0.6730	0.2941	0.6228	0.4753	0.0000	0.3623	0.8647	0.3889	0.8516
LEI228	0.7850	0.4500	0.7652	0.6275	0.4500	0.5541	0.7941	0.2941	0.7646	0.6990	0.2353	0.6607	0.8677	0.3649	0.8553
LEI229	0.8663	0.4000	0.8531	0.6988	0.2000	0.6435	0.3642	**0.1111**	0.3267	0.5190	0.1176	0.4661	0.8770	0.2133	0.8650
LEI232	0.9028	0.3889	0.8947	0.8071	0.2778	0.7878	0.7299	0.1667	0.6978	**0.8827**	0.5556	**0.8717**	0.9127	0.3472	0.9064
LEI234	0.8163	**0.9000**	0.7930	0.7188	0.6500	0.6765	0.8056	0.6111	0.7821	0.8364	0.5556	0.8169	0.9237	**0.6842**	0.9186
LEI237	0.9155	0.3684	0.9094	0.8629	0.6842	0.8494	0.8704	**0.8333**	0.8582	0.7630	0.5882	0.7318	0.9431	0.6164	0.9403
LEI243	0.8925	0.3500	0.8833	0.6188	0.0500	0.5837	0.6790	0.6111	0.6233	0.8611	0.5000	0.8470	0.9194	0.3684	0.9140
LEI246	0.8767	0.0526	0.8640	0.7756	0.4737	0.7510	0.6713	0.2778	0.6418	0.6605	0.7222	0.6244	0.9043	0.3784	0.8968
LEI248	0.7654	0.3889	0.7302	0.6950	0.0000	0.6428	0.7299	0.5556	0.6932	0.8040	0.6667	0.7766	0.7941	0.3919	0.7667
MCW111	0.6065	0.6111	**0.5313**	0.5150	0.5000	0.4244	0.6235	0.3889	0.5443	0.5262	0.3889	0.4500	**0.7285**	0.4730	**0.6814**
Mean	**0.7975**	**0.4565**	**0.7716**	**0.6946**	**0.3822**	**0.6576**	**0.7396**	**0.4492**	**0.7112**	**0.6958**	**0.4550**	**0.6584**	**0.8830**	**0.4344**	**0.8715**

Table 4.3. Frequency of different alleles for microsatellite locus **ADL034** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
113	0.0000	0.0000	0.0000	0.0882	0.0224
121	0.0000	0.0000	0.0278	0.2353	0.0672
123	0.0000	0.0000	0.1667	0.0294	0.0522
125	0.0000	0.0526	0.0000	0.0000	0.0149
127	0.0385	0.0526	0.2500	0.0000	0.0896
129	0.0769	0.1053	0.0556	0.0000	0.0597
131	0.3462	0.4737	0.0000	0.0000	0.2015
133	0.2692	0.0000	0.0278	0.0000	0.0597
135	0.1154	0.0789	0.0278	0.0000	0.0522
137	0.0000	0.0000	0.0833	0.0588	0.0373
139	0.0385	0.0000	0.0278	0.5588	0.1567
141	0.0769	0.0000	0.0278	0.0000	0.0224
143	0.0385	0.0263	0.0833	0.0000	0.0373
147	0.0000	0.1842	0.0278	0.0000	0.0597
149	0.0000	0.0000	0.1667	0.0000	0.0448
151	0.0000	0.0263	0.0000	0.0000	0.0075
153	0.0000	0.0000	0.0000	0.0294	0.0075
159	0.0000	0.0000	0.0278	0.0000	0.0075
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.4 Frequency of different alleles for microsatellite locus **ADL120** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
146	0.0000	0.0000	0.0882	0.0000	0.0203
148	0.0000	0.0000	0.0882	0.0000	0.0203
150	0.0000	0.0000	0.4118	0.0000	0.0946
152	0.0000	0.0000	0.0882	1.0000	0.2635
156	0.3158	1.0000	0.0000	0.0000	0.3514
168	0.0000	0.0000	0.0588	0.0000	0.0135
170	0.0000	0.0000	0.0588	0.0000	0.0135
172	0.0000	0.0000	0.1471	0.0000	0.0338
174	0.0000	0.0000	0.0588	0.0000	0.0135
178	0.5000	0.0000	0.0000	0.0000	0.1284
182	0.1053	0.0000	0.0000	0.0000	0.0270
192	0.0789	0.0000	0.0000	0.0000	0.0203
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.5. Frequency of different alleles for microsatellite locus **ADL158** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
172	0.0263	0.0000	0.0000	0.0000	0.0068
174	0.0263	0.0000	0.0000	0.0000	0.0068
176	0.0789	0.0000	0.0000	0.0000	0.0205
180	0.1053	0.1500	0.0000	0.0000	0.0685
182	0.2632	0.0500	0.1471	0.0000	0.1164
184	0.1579	0.0750	0.2059	0.0588	0.1233
186	0.1842	0.1000	0.1765	0.0000	0.1164
188	0.0263	0.3000	0.2353	0.1176	0.1712
190	0.0000	0.1000	0.0588	0.2059	0.0890
194	0.1053	0.0250	0.0000	0.3824	0.1233
198	0.0263	0.0750	0.0882	0.0000	0.0479
204	0.0000	0.1250	0.0882	0.0882	0.0753
208	0.0000	0.0000	0.0000	0.1471	0.0342
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.6. Frequency of different alleles for microsatellite locus **ADL209** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
132	0.0000	0.0000	0.0000	0.0417	0.0086
134	0.4643	0.0000	0.0667	0.1667	0.1638
136	0.3571	0.0000	0.0333	0.2917	0.1552
138	0.0000	0.0000	0.0333	0.0000	0.0086
140	0.0000	0.0000	0.1000	0.0000	0.0259
154	0.1071	0.1471	0.0333	0.0417	0.0862
156	0.0000	0.0882	0.0667	0.0833	0.0603
158	0.0000	0.0000	0.0000	0.0417	0.0086
160	0.0714	0.0000	0.0000	0.0000	0.0172
166	0.0000	0.0000	0.0000	0.0417	0.0086
170	0.0000	0.0000	0.2333	0.1250	0.0862
172	0.0000	0.4412	0.1333	0.0000	0.1638
174	0.0000	0.2353	0.2333	0.0833	0.1466
176	0.0000	0.0882	0.0667	0.0417	0.0517
178	0.0000	0.0000	0.0000	0.0417	0.0086
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.7. Frequency of different alleles for microsatellite locus **ADL254** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
126	0.0000	0.0000	0.0278	0.0882	0.0286
128	0.0000	0.0250	0.0000	0.0294	0.0143
130	0.0667	0.0000	0.0000	0.0294	0.0214
132	0.1667	0.1000	0.0000	0.0000	0.0643
134	0.0667	0.1500	0.0278	0.0000	0.0643
136	0.1000	0.0250	0.0000	0.0000	0.0286
140	0.1333	0.0500	0.0000	0.1765	0.0857
142	0.0000	0.1250	0.0833	0.1176	0.0857
144	0.1667	0.0500	0.2222	0.1471	0.1429
146	0.1333	0.2250	0.0556	0.0294	0.1143
148	0.0000	0.2250	0.1667	0.0000	0.1071
150	0.1667	0.0250	0.0278	0.0000	0.0500
152	0.0000	0.0000	0.0278	0.0000	0.0071
156	0.0000	0.0000	0.0278	0.0000	0.0071
158	0.0000	0.0000	0.0278	0.0882	0.0286
160	0.0000	0.0000	0.0278	0.1471	0.0429
162	0.0000	0.0000	0.0000	0.0294	0.0071
164	0.0000	0.0000	0.0278	0.1176	0.0357
166	0.0000	0.0000	0.0278	0.0000	0.0071
168	0.0000	0.0000	0.0556	0.0000	0.0143
170	0.0000	0.0000	0.0833	0.0000	0.0214
172	0.0000	0.0000	0.0278	0.0000	0.0071
176	0.0000	0.0000	0.0278	0.0000	0.0071
180	0.0000	0.0000	0.0278	0.0000	0.0071
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.8. Frequency of different alleles for microsatellite locus **ADL265** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
100	0.0000	0.0000	0.1944	0.0000	0.0467
102	0.0000	0.0000	0.3333	0.2500	0.1400
104	0.0000	0.0250	0.3611	0.4444	0.2000
106	0.1579	0.0250	0.0000	0.0000	0.0467
108	0.0263	0.0000	0.0000	0.0000	0.0067
110	0.0526	0.3000	0.0000	0.0000	0.0933
112	0.1842	0.2500	0.0000	0.0000	0.1133
114	0.5263	0.0000	0.0000	0.0000	0.1333
120	0.0000	0.0000	0.0833	0.1944	0.0667
124	0.0000	0.0000	0.0278	0.1111	0.0333
128	0.0263	0.0000	0.0000	0.0000	0.0067
130	0.0000	0.1500	0.0000	0.0000	0.0400
134	0.0263	0.2000	0.0000	0.0000	0.0600
136	0.0000	0.0250	0.0000	0.0000	0.0067
144	0.0000	0.0250	0.0000	0.0000	0.0067
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.9. Frequency of different alleles for microsatellite locus **ADL270** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
86	0.0000	0.0500	0.0000	0.0625	0.0278
90	0.1053	0.0000	0.0000	0.0000	0.0278
100	0.4211	0.5750	0.2353	0.0000	0.3264
102	0.2368	0.1250	0.2059	0.0000	0.1458
106	0.0526	0.0000	0.0000	0.0000	0.0139
108	0.0789	0.0000	0.0000	0.2813	0.0833
110	0.1053	0.0000	0.0000	0.4375	0.1250
112	0.0000	0.1000	0.0000	0.0625	0.0417
114	0.0000	0.0250	0.1471	0.0000	0.0417
116	0.0000	0.0000	0.1176	0.0000	0.0278
120	0.0000	0.0250	0.0294	0.0000	0.0139
122	0.0000	0.0000	0.1176	0.0625	0.0417
124	0.0000	0.0500	0.0294	0.0000	0.0208
126	0.0000	0.0000	0.0588	0.0000	0.0139
128	0.0000	0.0500	0.0588	0.0000	0.0278
130	0.0000	0.0000	0.0000	0.0313	0.0069
132	0.0000	0.0000	0.0000	0.0625	0.0139
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.10. Frequency of different alleles for microsatellite locus **ADL327** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
98	0.0000	0.0000	0.0000	0.0882	0.0205
100	0.0000	0.0000	0.0000	0.0294	0.0068
102	0.0000	0.0000	0.0278	0.0000	0.0068
104	0.0000	0.0526	0.1389	0.0000	0.0479
106	0.0000	0.5000	0.0556	0.0000	0.1438
110	0.0000	0.0000	0.0000	0.3529	0.0822
112	0.2105	0.0263	0.0278	0.3824	0.1575
114	0.4474	0.0263	0.0000	0.0588	0.1370
116	0.2368	0.0263	0.0000	0.0000	0.0685
120	0.0000	0.0000	0.0000	0.0294	0.0068
122	0.0000	0.0000	0.1111	0.0000	0.0274
124	0.0000	0.0000	0.0000	0.0294	0.0068
126	0.0526	0.0000	0.0000	0.0000	0.0137
128	0.0263	0.0000	0.6111	0.0000	0.1575
130	0.0000	0.0000	0.0278	0.0000	0.0068
132	0.0263	0.3421	0.0000	0.0000	0.0959
134	0.0000	0.0263	0.0000	0.0294	0.0137
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.11. Frequency of different alleles for microsatellite locus **ADL331** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
151	0.0000	0.2000	0.0000	0.2778	0.1233
153	0.0000	0.1750	0.0833	0.0833	0.0890
155	0.0000	0.1500	0.1111	0.0278	0.0753
157	0.0000	0.1000	0.1389	0.2222	0.1164
159	0.5882	0.0750	0.0000	0.0000	0.1575
161	0.1471	0.0000	0.0000	0.0000	0.0342
163	0.1471	0.0500	0.0000	0.0000	0.0479
169	0.0000	0.0250	0.0000	0.0000	0.0068
171	0.0000	0.0000	0.0556	0.0556	0.0274
173	0.0000	0.0000	0.0278	0.0000	0.0068
175	0.0000	0.0500	0.0278	0.0278	0.0274
177	0.0000	0.0500	0.0278	0.0000	0.0205
179	0.0000	0.1250	0.0000	0.0278	0.0411
181	0.0000	0.0000	0.0000	0.1111	0.0274
183	0.0000	0.0000	0.0556	0.0000	0.0137
185	0.0588	0.0000	0.1111	0.0556	0.0548
187	0.0000	0.0000	0.2500	0.0278	0.0685
189	0.0294	0.0000	0.0278	0.0278	0.0205
191	0.0294	0.0000	0.0278	0.0000	0.0137
195	0.0000	0.0000	0.0000	0.0278	0.0068
197	0.0000	0.0000	0.0556	0.0278	0.0205
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.12. Frequency of different alleles for microsatellite locus **LEI194** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
118	0.0500	0.0000	0.0000	0.0000	0.0133
120	0.1000	0.0750	0.0000	0.0000	0.0467
124	0.2000	0.4750	0.0000	0.0000	0.1800
128	0.2000	0.0250	0.1176	0.0556	0.1000
132	0.0000	0.0500	0.0294	0.0000	0.0200
136	0.0500	0.0000	0.0294	0.3333	0.1000
138	0.0500	0.0000	0.0000	0.0000	0.0133
140	0.0000	0.0000	0.2647	0.1944	0.1067
144	0.0000	0.0000	0.0294	0.0000	0.0067
150	0.0000	0.0250	0.0000	0.0000	0.0067
156	0.0000	0.2250	0.0000	0.0000	0.0600
160	0.0000	0.0000	0.0294	0.3889	0.1000
164	0.0000	0.1000	0.4118	0.0278	0.1267
166	0.0000	0.0000	0.0882	0.0000	0.0200
168	0.0000	0.0250	0.0000	0.0000	0.0067
182	0.1250	0.0000	0.0000	0.0000	0.0333
184	0.2250	0.0000	0.0000	0.0000	0.0600
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.13. Frequency of different alleles for microsatellite locus **LEI209** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
136	0.0250	0.0000	0.0000	0.0000	0.0066
140	0.0500	0.0000	0.0000	0.5278	0.1382
142	0.0000	0.0750	0.0000	0.0000	0.0197
144	0.0000	0.2500	0.0000	0.2500	0.1250
148	0.0000	0.0500	0.0000	0.0278	0.0197
152	0.1500	0.0000	0.3333	0.0278	0.1250
156	0.1500	0.0000	0.1944	0.0000	0.0855
160	0.1000	0.0000	0.1389	0.0000	0.0592
162	0.0250	0.0000	0.0000	0.0000	0.0066
164	0.2500	0.0000	0.0556	0.0000	0.0789
168	0.1500	0.0000	0.0000	0.0556	0.0526
172	0.0250	0.2750	0.0000	0.0833	0.0987
176	0.0750	0.3250	0.0278	0.0278	0.1184
180	0.0000	0.0250	0.1111	0.0000	0.0329
184	0.0000	0.0000	0.0278	0.0000	0.0066
188	0.0000	0.0000	0.0556	0.0000	0.0132
192	0.0000	0.0000	0.0278	0.0000	0.0066
196	0.0000	0.0000	0.0278	0.0000	0.0066
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.14. Frequency of different alleles for microsatellite locus **LEI229** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
193	0.0000	0.0000	0.0000	0.6471	0.1467
211	0.0000	0.0000	0.0556	0.0000	0.0133
220	0.0750	0.0000	0.0000	0.0000	0.0200
238	0.0750	0.0000	0.0000	0.0000	0.0200
247	0.1250	0.0000	0.1667	0.0000	0.0733
256	0.0750	0.0000	0.0000	0.0000	0.0200
265	0.0250	0.0000	0.7778	0.0000	0.1933
274	0.2500	0.0000	0.0000	0.0000	0.0667
283	0.1000	0.1500	0.0000	0.0000	0.0667
292	0.1000	0.3750	0.0000	0.2353	0.1800
301	0.0500	0.1250	0.0000	0.0588	0.0600
310	0.0000	0.3500	0.0000	0.0588	0.1067
355	0.1250	0.0000	0.0000	0.0000	0.0333
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.15. Frequency of different alleles for microsatellite locus **LEI212** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
294	0.0263	0.0000	0.0000	0.0000	0.0069
322	0.0000	0.0000	0.0278	0.0000	0.0069
328	0.0000	0.2353	0.0556	0.0000	0.0694
332	0.0000	0.1471	0.1667	0.0000	0.0764
336	0.0000	0.1471	0.0000	0.0000	0.0347
342	0.0000	0.0294	0.0000	0.0000	0.0069
360	0.0000	0.0000	0.0278	0.5556	0.1458
362	0.0263	0.0000	0.0000	0.0000	0.0069
370	0.0000	0.0000	0.1944	0.0000	0.0486
372	0.0263	0.0000	0.0000	0.0000	0.0069
376	0.0526	0.0000	0.0000	0.0000	0.0139
378	0.0263	0.0000	0.0000	0.0000	0.0069
382	0.0789	0.0000	0.0000	0.0000	0.0208
384	0.0526	0.0000	0.0000	0.0000	0.0139
386	0.0000	0.0000	0.0556	0.0000	0.0139
388	0.0000	0.0000	0.0000	0.0278	0.0069
390	0.0526	0.0000	0.0556	0.0000	0.0278
392	0.0000	0.0000	0.1667	0.0556	0.0556
394	0.0263	0.0000	0.0000	0.0000	0.0069
396	0.0526	0.0000	0.0000	0.0000	0.0139
402	0.0263	0.0000	0.0000	0.0000	0.0069
404	0.0263	0.0000	0.0000	0.0000	0.0069
406	0.0263	0.0000	0.0000	0.0000	0.0069
408	0.0789	0.0000	0.0000	0.0000	0.0208
412	0.0000	0.0000	0.0000	0.3333	0.0833
414	0.1316	0.0000	0.0000	0.0000	0.0347
422	0.0000	0.0000	0.0278	0.0000	0.0069
424	0.0526	0.0000	0.0000	0.0000	0.0139
426	0.0263	0.0000	0.0278	0.0000	0.0139
428	0.0263	0.0000	0.0000	0.0278	0.0139
430	0.0263	0.0000	0.0278	0.0000	0.0139
432	0.0000	0.0588	0.0000	0.0000	0.0139
434	0.0263	0.0000	0.0000	0.0000	0.0069
436	0.0263	0.0588	0.0000	0.0000	0.0208
440	0.0789	0.0000	0.0000	0.0000	0.0208
444	0.0263	0.0000	0.0000	0.0000	0.0069
446	0.0000	0.0294	0.0556	0.0000	0.0208
448	0.0000	0.0294	0.0278	0.0000	0.0139
452	0.0000	0.2059	0.0000	0.0000	0.0486
456	0.0000	0.0294	0.0000	0.0000	0.0069
458	0.0000	0.0000	0.0556	0.0000	0.0139
484	0.0000	0.0294	0.0000	0.0000	0.0069
492	0.0000	0.0000	0.0278	0.0000	0.0069
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.16. Frequency of different alleles for microsatellite locus **LEI214** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
134	0.0000	0.0000	0.0000	0.0588	0.0137
140	0.0000	0.0000	0.8333	0.0000	0.2055
144	0.0789	0.0263	0.0000	0.0000	0.0274
148	0.3421	0.0000	0.0000	0.0000	0.0890
156	0.0000	0.0000	0.0000	0.3529	0.0822
164	0.0000	0.5789	0.0556	0.0000	0.1644
168	0.0526	0.2368	0.0000	0.0000	0.0753
172	0.0263	0.0000	0.0000	0.0000	0.0068
184	0.0263	0.0000	0.0000	0.0000	0.0068
186	0.0263	0.0000	0.0000	0.0000	0.0068
188	0.0263	0.0000	0.0000	0.0000	0.0068
192	0.0526	0.0000	0.0000	0.0000	0.0137
196	0.0526	0.0000	0.0000	0.0000	0.0137
200	0.0526	0.0000	0.0000	0.0000	0.0137
204	0.1842	0.0000	0.0000	0.0000	0.0479
208	0.0526	0.0000	0.0000	0.0000	0.0137
224	0.0263	0.0000	0.0000	0.0000	0.0068
272	0.0000	0.1053	0.0278	0.0000	0.0342
280	0.0000	0.0526	0.0000	0.1176	0.0411
284	0.0000	0.0000	0.0833	0.0882	0.0411
288	0.0000	0.0000	0.0000	0.1471	0.0342
308	0.0000	0.0000	0.0000	0.0882	0.0205
312	0.0000	0.0000	0.0000	0.0882	0.0205
332	0.0000	0.0000	0.0000	0.0588	0.0137
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.17. Frequency of different alleles for microsatellite locus **LEI228** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
190	0.0250	0.0000	0.0000	0.0000	0.0068
194	0.1250	0.4250	0.0000	0.4706	0.2568
198	0.0000	0.0250	0.0000	0.0000	0.0068
202	0.0750	0.0250	0.2353	0.1176	0.1081
206	0.0250	0.0000	0.2647	0.0588	0.0811
210	0.1500	0.0000	0.2647	0.0000	0.1014
214	0.4000	0.0000	0.0294	0.0000	0.1149
218	0.0500	0.0000	0.0294	0.0588	0.0338
222	0.0000	0.0000	0.0294	0.0000	0.0068
226	0.0250	0.0000	0.0588	0.2353	0.0743
230	0.0750	0.0000	0.0588	0.0588	0.0473
234	0.0000	0.4250	0.0294	0.0000	0.1216
238	0.0000	0.1000	0.0000	0.0000	0.0270
254	0.0250	0.0000	0.0000	0.0000	0.0068
266	0.0250	0.0000	0.0000	0.0000	0.0068
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.18. Frequency of different alleles for microsatellite locus **LEI217** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
148	0.0000	0.3750	0.0000	0.0000	0.1000
156	0.0526	0.0250	0.0000	0.0000	0.0200
158	0.0263	0.0000	0.0000	0.0000	0.0067
160	0.0263	0.0000	0.0000	0.0000	0.0067
164	0.1053	0.0000	0.0000	0.0000	0.0267
168	0.0526	0.0000	0.0000	0.0000	0.0133
172	0.0526	0.0000	0.0000	0.0000	0.0133
176	0.0263	0.0000	0.0000	0.0000	0.0067
180	0.0263	0.0000	0.0000	0.0000	0.0067
188	0.1579	0.0000	0.0000	0.0000	0.0400
192	0.0000	0.0000	0.0000	0.0556	0.0133
196	0.0000	0.2000	0.0000	0.0556	0.0667
200	0.0000	0.2500	0.0278	0.2222	0.1267
204	0.0000	0.1250	0.1111	0.1111	0.0867
212	0.0263	0.0000	0.0833	0.0278	0.0333
216	0.0263	0.0000	0.1111	0.0833	0.0533
224	0.0263	0.0000	0.1667	0.0278	0.0533
228	0.0263	0.0000	0.3611	0.1111	0.1200
232	0.0526	0.0000	0.0556	0.0000	0.0267
236	0.0789	0.0000	0.0000	0.1111	0.0467
240	0.0263	0.0000	0.0000	0.1667	0.0467
248	0.0263	0.0000	0.0000	0.0278	0.0133
252	0.0000	0.0000	0.0278	0.0000	0.0067
256	0.0263	0.0000	0.0278	0.0000	0.0133
260	0.0263	0.0000	0.0000	0.0000	0.0067
264	0.0000	0.0000	0.0278	0.0000	0.0067
268	0.0789	0.0000	0.0000	0.0000	0.0200
272	0.0000	0.0250	0.0000	0.0000	0.0067
300	0.0526	0.0000	0.0000	0.0000	0.0133
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.19. Frequency of different alleles for microsatellite locus **MCW 111** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
98	0.0000	0.0500	0.4444	0.3056	0.1959
100	0.3611	0.6000	0.3889	0.0833	0.3649
102	0.0278	0.3500	0.1667	0.6111	0.2905
104	0.5000	0.0000	0.0000	0.0000	0.1216
106	0.1111	0.0000	0.0000	0.0000	0.0270
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.20. Frequency of different alleles for microsatellite locus **LEI248** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
222	0.0000	0.0000	0.0278	0.0000	0.0068
226	0.0278	0.0000	0.0000	0.0556	0.0203
230	0.0278	0.0000	0.0000	0.0556	0.0203
234	0.1944	0.0000	0.0000	0.0556	0.0608
238	0.2778	0.1000	0.0278	0.2778	0.1689
242	0.3333	0.4000	0.2778	0.2500	0.3176
246	0.0556	0.3500	0.4167	0.1667	0.2500
250	0.0278	0.1000	0.0833	0.1389	0.0878
254	0.0556	0.0500	0.0278	0.0000	0.0338
258	0.0000	0.0000	0.0833	0.0000	0.0203
262	0.0000	0.0000	0.0556	0.0000	0.0135
Mean	1.0000	1.0000	1.0000	1.0000	1.0000

Table 4.21. Frequency of different alleles for microsatellite locus **LEI221** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
120	0.0263	0.0000	0.0000	0.0000	0.0069
160	0.0526	0.0000	0.0000	0.0000	0.0139
164	0.0263	0.0000	0.0000	0.0000	0.0069
172	0.0263	0.0000	0.0000	0.3889	0.1042
176	0.0263	0.0000	0.0294	0.6111	0.1667
180	0.0526	0.1111	0.0000	0.0000	0.0417
184	0.1316	0.0278	0.2941	0.0000	0.1111
188	0.0263	0.0556	0.1176	0.0000	0.0486
192	0.2632	0.2778	0.4706	0.0000	0.2500
196	0.1579	0.3056	0.0294	0.0000	0.1250
200	0.0526	0.0556	0.0000	0.0000	0.0278
204	0.0526	0.0000	0.0000	0.0000	0.0139
208	0.0263	0.0000	0.0000	0.0000	0.0069
212	0.0000	0.0000	0.0588	0.0000	0.0139
216	0.0263	0.0000	0.0000	0.0000	0.0069
220	0.0263	0.0000	0.0000	0.0000	0.0069
224	0.0263	0.0278	0.0000	0.0000	0.0139
232	0.0000	0.0556	0.0000	0.0000	0.0139
236	0.0000	0.0278	0.0000	0.0000	0.0069
240	0.0000	0.0278	0.0000	0.0000	0.0069
244	0.0000	0.0278	0.0000	0.0000	0.0069
Mean	1.0000	1.0000	1.0000	1.0000	1.0000

Table 4.22. Frequency of different alleles for microsatellite locus **LEI246** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	**WLH**	**AS**	**RC**	**Overall**
199	0.0000	0.0000	0.0000	0.1667	0.0405
203	0.0000	0.3947	0.0556	0.1667	0.1554
211	0.0526	0.0000	0.0000	0.5278	0.1419
215	0.0526	0.0000	0.5278	0.0556	0.1554
219	0.1579	0.0000	0.1667	0.0000	0.0811
223	0.1316	0.0000	0.0000	0.0278	0.0405
227	0.1579	0.0000	0.0278	0.0278	0.0541
231	0.1579	0.0000	0.0556	0.0000	0.0541
235	0.0000	0.0789	0.1111	0.0000	0.0473
239	0.0526	0.0000	0.0000	0.0278	0.0203
243	0.1316	0.0000	0.0000	0.0000	0.0338
247	0.0000	0.0526	0.0000	0.0000	0.0135
255	0.0526	0.1053	0.0556	0.0000	0.0541
259	0.0526	0.1842	0.0000	0.0000	0.0608
263	0.0000	0.0263	0.0000	0.0000	0.0068
267	0.0000	0.0526	0.0000	0.0000	0.0135
271	0.0000	0.1053	0.0000	0.0000	0.0270
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.23. Frequency of different alleles for microsatellite locus **LEI232** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	**WLH**	**AS**	**RC**	**Overall**
178	0.0000	0.0000	0.0000	0.0833	0.0208
180	0.0556	0.0000	0.0000	0.1389	0.0486
184	0.0278	0.0000	0.0000	0.0000	0.0069
186	0.1389	0.0000	0.0000	0.0278	0.0417
188	0.0000	0.0000	0.1111	0.0556	0.0417
190	0.1389	0.0000	0.4444	0.0556	0.1597
194	0.0000	0.0556	0.1944	0.1944	0.1111
196	0.0833	0.0000	0.1111	0.0556	0.0625
200	0.0000	0.1389	0.0000	0.0833	0.0556
202	0.0000	0.0556	0.0000	0.0000	0.0139
204	0.0833	0.1111	0.0000	0.0278	0.0556
206	0.0556	0.0000	0.0000	0.0000	0.0139
208	0.0278	0.0000	0.0833	0.0000	0.0278
212	0.0833	0.3611	0.0000	0.0278	0.1181
216	0.1389	0.1389	0.0556	0.1667	0.1250
220	0.0833	0.0000	0.0000	0.0833	0.0417
228	0.0556	0.0000	0.0000	0.0000	0.0139
232	0.0278	0.0000	0.0000	0.0000	0.0069
276	0.0000	0.0556	0.0000	0.0000	0.0139
280	0.0000	0.0278	0.0000	0.0000	0.0069
284	0.0000	0.0278	0.0000	0.0000	0.0069
290	0.0000	0.0278	0.0000	0.0000	0.0069
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.24. Frequency of different alleles for microsatellite locus **LEI234** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
216	0.0000	0.0000	0.0833	0.0000	0.0197
220	0.0000	0.0000	0.3333	0.0833	0.0987
224	0.0500	0.4000	0.0000	0.0000	0.1184
228	0.1000	0.0250	0.0000	0.0000	0.0329
232	0.3000	0.0000	0.0000	0.0000	0.0789
260	0.0000	0.0000	0.0000	0.0556	0.0132
264	0.1250	0.0000	0.0000	0.0000	0.0329
268	0.2000	0.0000	0.0000	0.0000	0.0526
272	0.1500	0.0000	0.0000	0.0000	0.0395
276	0.0000	0.0000	0.0000	0.2500	0.0592
280	0.0000	0.0000	0.1111	0.2222	0.0789
284	0.0000	0.0500	0.0556	0.0000	0.0263
288	0.0250	0.3250	0.1389	0.0000	0.1250
292	0.0000	0.0250	0.0556	0.0000	0.0197
296	0.0000	0.0750	0.0000	0.0000	0.0197
300	0.0000	0.0250	0.1944	0.1667	0.0921
304	0.0500	0.0750	0.0278	0.0833	0.0592
328	0.0000	0.0000	0.0000	0.0278	0.0066
364	0.0000	0.0000	0.0000	0.0556	0.0132
388	0.0000	0.0000	0.0000	0.0556	0.0132
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.25. Frequency of different alleles for microsatellite locus **LEI192** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
255	0.0000	0.5500	0.1389	0.3611	0.2703
266	0.0000	0.3000	0.0000	0.3611	0.1689
270	0.0000	0.1500	0.0000	0.0000	0.0405
274	0.2778	0.0000	0.0000	0.0000	0.0676
283	0.0000	0.0000	0.0556	0.0000	0.0135
286	0.0000	0.0000	0.0833	0.0000	0.0203
287	0.0000	0.0000	0.0556	0.0000	0.0135
303	0.1111	0.0000	0.3056	0.0000	0.1014
323	0.0000	0.0000	0.2778	0.1111	0.0946
332	0.0000	0.0000	0.0278	0.0000	0.0068
347	0.0000	0.0000	0.0000	0.1667	0.0405
370	0.2222	0.0000	0.0000	0.0000	0.0541
393	0.1111	0.0000	0.0000	0.0000	0.0270
401	0.1667	0.0000	0.0000	0.0000	0.0405
417	0.0000	0.0000	0.0556	0.0000	0.0135
465	0.1111	0.0000	0.0000	0.0000	0.0270
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.26. Frequency of different alleles for microsatellite locus **LEI237** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	**WLH**	**AS**	**RC**	**Overall**
211	0.0000	0.1053	0.0000	0.0000	0.0274
215	0.0000	0.2105	0.0000	0.0000	0.0548
219	0.0000	0.0263	0.0000	0.0000	0.0068
279	0.0000	0.0000	0.1111	0.0000	0.0274
283	0.0526	0.0000	0.0000	0.0000	0.0137
287	0.1053	0.0000	0.0000	0.0000	0.0274
291	0.0000	0.0526	0.0000	0.0000	0.0137
295	0.0789	0.0000	0.0833	0.0000	0.0411
299	0.1053	0.0000	0.2500	0.0000	0.0890
303	0.0789	0.0000	0.0000	0.0000	0.0205
307	0.1579	0.0000	0.0278	0.1471	0.0822
311	0.0000	0.0000	0.1111	0.0882	0.0479
315	0.0526	0.0000	0.0000	0.0588	0.0274
319	0.0000	0.0000	0.0278	0.0294	0.0137
323	0.0000	0.0789	0.1111	0.2353	0.1027
327	0.0526	0.0000	0.1111	0.0294	0.0479
331	0.0526	0.0000	0.0556	0.0000	0.0274
335	0.0789	0.0000	0.0000	0.0000	0.0205
343	0.0526	0.0526	0.0000	0.0000	0.0274
347	0.0263	0.0263	0.0000	0.0000	0.0137
351	0.0526	0.0000	0.0000	0.3824	0.1027
355	0.0263	0.0000	0.0000	0.0294	0.0137
359	0.0263	0.0000	0.0556	0.0000	0.0205
363	0.0000	0.0526	0.0000	0.0000	0.0137
375	0.0000	0.0263	0.0556	0.0000	0.0205
383	0.0000	0.2368	0.0000	0.0000	0.0616
387	0.0000	0.0789	0.0000	0.0000	0.0205
391	0.0000	0.0526	0.0000	0.0000	0.0137
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.27. Frequency of different alleles for microsatellite locus **LEI243** in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC)

Allele sizes (bps)	Allelic frequency				
	RJF	WLH	AS	RC	Overall
170	0.0000	0.0000	0.0278	0.0000	0.0066
172	0.0750	0.0000	0.0000	0.0000	0.0197
176	0.0000	0.0000	0.0000	0.0278	0.0066
178	0.0000	0.0000	0.0278	0.0278	0.0132
180	0.0000	0.0500	0.4167	0.1111	0.1382
182	0.0250	0.0000	0.0000	0.0000	0.0066
184	0.0000	0.1500	0.0556	0.0000	0.0526
186	0.0000	0.5750	0.0000	0.0000	0.1513
188	0.0000	0.1500	0.0000	0.0833	0.0592
190	0.0500	0.0000	0.0000	0.0000	0.0132
192	0.0500	0.0500	0.0000	0.0278	0.0329
196	0.0750	0.0000	0.1111	0.0000	0.0461
200	0.0000	0.0250	0.3611	0.0833	0.1118
204	0.1500	0.0000	0.0000	0.0556	0.0526
208	0.1000	0.0000	0.0000	0.0000	0.0263
304	0.2000	0.0000	0.0000	0.0833	0.0724
308	0.0000	0.0000	0.0000	0.2222	0.0526
312	0.0000	0.0000	0.0000	0.2222	0.0526
316	0.0500	0.0000	0.0000	0.0000	0.0132
320	0.1000	0.0000	0.0000	0.0000	0.0263
324	0.0500	0.0000	0.0000	0.0278	0.0197
328	0.0500	0.0000	0.0000	0.0000	0.0132
332	0.0250	0.0000	0.0000	0.0000	0.0066
364	0.0000	0.0000	0.0000	0.0278	0.0066
Mean	**1.0000**	**1.0000**	**1.0000**	**1.0000**	**1.0000**

Table 4.28. Population-specific alleles in RJF, White Leghorn (WLH), Aseel (AS) and Red Cornish (RC) at various microsatellite loci

Locus	Population specific alleles			
	RJF	**WLH**	**Aseel**	**RC**
ADL034	-	125(0.0526) 151(0.0263)	149(0.1667) 159(0.0278)	113(0.0882) 153(0.0294)
ADL120	178(0.5000) 182(0.1053) 192(0.0789)	-	146(0.0882) 148(0.0882) 150(0.4118) 168(0.0588) 170(0.0558) 172(0.1471) 174(0.0558)	-
ADL158	172(0.0263) 174(0.0263) 176(0.0789)	-	-	208(0.1471)
ADL209	160(0.0714)	-	138(0.0333) 140(0.1000)	132(0.0417) 158(0.0417) 166(0.0417) 178(0.0417)
ADL254	-	-	152(0.0278) 156(0.0278) 166(0.0278) 168(0.0556) 170(0.0833) 172(0.0278) 176(0.0278) 180(0.0278)	162(0.0294)
ADL265	108(0.0263) 114(0.5263) 128(0.0263)	130(0.1500) 136(0.0250) 144(0.0250)	100(0.1944)	-
ADL270	90(0.1053) 106(0.0526)	-	116(0.1176) 126(0.0588)	130(0.0313) 132(0.0625)
ADL327	126(0.0526)	-	102(0.0278) 122(0.1111) 130(0.0278)	100(0.0294) 110(0.3529) 120(0.0294) 124(0.0294) 98(0.0882)
ADL331	161(0.1471)	169(0.0250)	173(0.0278) 183(0.0556)	181(0.1111) 195(0.0278)
LEI194	118(0.0500) 138(0.0500) 182(0.1250)	150(0.0250) 156(0.0250) 168(0.0250)	144(0.0294) 166(0.0882)	-

	184(0.2250)			
LEI209	136(0.0250) 162(0.0250)	142(0.0750)	184(0.0278) 188(0.0556) 192(0.0278) 196(0.0278)	-
LEI212	294(0.0263) 362(0.0263) 372(0.0263) 376(0.0526) 378(0.0263) 382(0.0789) 384(0.0526) 394(0.0263) 396(0.0526) 402(0.0263) 404(0.0263) 406(0.0263) 408(0.0789) 414(0.1316) 424(0.0526) 434(0.0263) 440(0.0789) 444(0.0263)	336(0.1471) 342(0.0294) 432(0.0588) 452(0.20590 456(0.0294) 484(0.0294)	322(0.0278) 370(0.1944) 386(0.0556) 422(0.0278) 458(0.0556) 492(0.0278)	388(0.0278) 412(0.3333)
LEI214	148(0.3421) 172(0.0263) 184(0.0263) 186(0.0263) 188(0.0263) 192(0.0526) 196(0.0526) 200(0.0526) 204(0.1842) 208(0.0526) 224(0.0263)	-	140(0.8333)	134(0.0588) 156(0.3529) 288(0.1471) 308(0.0882) 312(0.0882) 332(0.0588)
LEI217	158 (0.0263) 160(0.0263) 164(0.1053) 168(0.0526) 172(0.0526) 176(0.0263) 180(0.0263) 188(0.1579) 260(0.0263) 268(0.0789)	148(0.3750) 272(0.0250)	252(0.0278) 264(0.0278)	192(0.0556)

	300(0.0526)			
LEI221	120(0.0263) 160(0.0526) 164(0.0263) 204(0.0526) 208(0.0263) 216(0.0263) 220(0.0263)	232(0.0526) 236(0.0278) 240(0.0278) 244(0.0278)	212(0.0588)	-
LEI228	190(0.0250) 254(0.0250) 266(0.0250)	198(0.0250) 238(0.1000)	222(0.0294)	-
LEI229	220(0.0750) 238(0.0750) 256(0.0750) 274(0.2500) 355(0.1250)	-	211(0.0526)	193(0.6471)
LEI232	184(0.0278) 206(0.0556) 228(0.0556) 232(0.0278)	202(0.0556) 276(0.0556) 280(0.0278) 284(0.0278) 290(0.0278)	-	178(0.0833)
LEI234	232(0.3000) 264(0.1250) 268(0.2000) 272(0.1500)	296(0.0750)	216(0.0833)	260(0.0556) 276(0.2500) 328(0.0278) 364(0.0556) 388(0.0556)
LEI237	283(0.0526) 287(0.1053) 303(0.0789) 335(0.0789)	211(0.1053) 215(0.2105) 219(0.0263) 291(0.0526) 363(0.0526) 383(0.0268) 387(0.0789) 391(0.0526)	279(0.1111)	-
LEI243	172(0.0750) 182(0.0250) 190(0.0500) 208(0.1000) 316(0.0500) 320(0.1000) 328(0.0500) 332(0.0250)	186(0.5750)	170(0.0278)	176(0.0278) 308(0.2222) 312(0.2222) 364(0.0278)
LEI246	243(0.1316)	247(0.0526) 263(0.0263)	-	199(0.1667)

		267(0.0526) 271(0.1053)		
LEI248			222(0.0278) 258(0.0833) 262(0.0556)	
LEI192	274(0.2778) 370(0.2222) 393(0.1111) 401(0.1667) 465(0.1111)	270(0.1500)	283(0.0556) 286(0.0833) 287(0.0556) 332(0.0278) 417(0.0556)	347(0.1667)
MCW111	104(0.5000) 106(0.5000)	-	-	-
Total	**103**	**44**	**56**	**39**

Table 4.29. F-Statistics and gene flow estimated using microsatellite markers at each locus across the populations

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
ADL034	0.3178	0.4355	0.1726	1.1982
ADL120	0.5580	**0.8024**	**0.5530**	**0.2021**
ADL158	0.4662	0.5096	0.0812	2.8305
ADL209	0.3184	0.4091	0.1330	1.6294
ADL254	0.3112	0.3511	**0.0580**	**4.0612**
ADL265	0.3888	0.5080	0.1951	1.0315
ADL270	0.4451	0.5215	0.1376	1.5670
ADL327	0.4005	0.5560	0.2594	0.7139
ADL331	0.5186	0.5806	0.1288	1.6906
LEI192	0.3455	0.4560	0.1689	1.2303
LEI194	0.3982	0.5022	0.1729	1.1958
LEI209	0.4179	0.5080	0.1547	1.3665
LEI212	0.3218	0.4165	0.1396	1.5413
LEI214	0.6286	0.7396	0.2987	0.5868
LEI217	0.1858	0.2692	0.1025	2.1892
LEI221	0.4567	0.5558	0.1823	1.1213
LEI228	0.5080	0.5886	0.1637	1.2771
LEI229	**0.6615**	0.7626	0.2987	0.5868
LEI232	0.5820	0.6196	0.0899	2.5300
LEI234	**0.1449**	**0.2649**	0.1403	1.5314
LEI237	0.2748	0.3431	0.0942	2.4036
LEI243	0.5048	0.5888	0.1697	1.2230
LEI246	0.4885	0.5775	0.1739	1.1875
LEI248	0.4620	0.4940	0.0596	3.9413
MCW111	0.1683	0.3547	0.2241	0.8658
Mean	**0.4047**	**0.5068**	**0.1716**	**1.2070**

Table 4.30. F- Statistics and Gene flow between populations based on all microsatellite markers

Populations	F_{IS}	F_{IT}	F_{ST}	Nm
RJF & WLH	0.4380	0.4963	0.1038	2.1593
RJF & AS	0.4108	0.4713	0.1027	**2.1840**
RJF & RC	0.3900	0.4647	0.1224	1.7921
WLH & AS	0.4205	0.4948	0.1283	1.6984
WLH & RC	0.3979	0.4848	**0.1443**	1.4822
AS & RC	0.3699	0.4504	0.1278	1.7057

Table 4.31. Genetic distance measures based on shared allele frequencies using Microsatellite markers between the populations

Population	RJF	WLH	AS	RC
RJF	-			
WLH	0.7562	-		
AS	0.7689	0.7495	-	
RC	0.8093	0.7637	0.7302	-

constructed among all 76 birds showed relationships among individuals of all chicken breeds **(Fig 4.26- B)**.

4.2. AFLP markers analysis

A total of 20 primer combinations using four *Eco*RI selective primers and six *Taq*I selective combination **(Table 3.2)** were used to detect polymorphism between RJF and three domestic chicken breeds namely as WLH, AS and RC. Each primer combination obtained large numbers of bands. The AFLP amplification profiles with different primer combinations, resolved on 3.5 % Metaphor agarose gel have been shown in **Fig 4.27-4.46**. Only intense and unambiguous AFLP bands found in each individual of all four populations were manually scored as dominant markers in binary matrices using values 1 and 0 (indicating band presence and absence respectively). Each AFLP band was treated as a separate putative locus with two alleles either 1 or 0.

4.2.1. Genetic diversity analysis

A total of 318 scorable AFLP bands in the range of 50-500 bp were detected across the populations. Out of which, 309 (97.17%) were polymorphic bands. The extant of polymorphism varied within the populations as well as with each primer combinations. Details are presented in **Table 4.32**. In RJF population, a total of 311 bands could be scored. Out of which, 302 (97%) were found polymorphic. Among the primer combinations the extant of polymorphism was 85% to 100%. In WLH population, a total of 270 bands could be scored. Out of which, 60 (22%) were found polymorphic. Among the primer combinations the extant of polymorphism was 0% to 52%. In AS population, a total of 282 bands could be scored. Out of which, 105 (37%) were found polymorphic. Among the primer combinations the extant of polymorphism was 11% to 63%. In RC population, a total of 281 bands could be scored. Out of which, 57 (20%) were found polymorphic. Among the primer combinations the extant of polymorphism was 0% to 50%.

The genetic diversity (h) within RJF and domestic chicken breeds and across populations was estimated at each locus as well as pooled over all the loci under different primer-combinations as per Nei's (1973) and presented in **Table 4.33 and 4.34**, respectively. The estimates showed wide variation among the chicken populations as well as at the loci/primer-combinations.

Shannon's Information Index (I) within RJF and domestic chicken populations and across populations was estimated at each locus as well as pooled over all the loci under different primer-combinations as per Shannon (1949) and presented in **Table 4.33 and 4.34**, respectively. The Shannon's Information Index estimates showed wide variation among the chicken populations as well as at the loci/primer-combinations.

Mean Nei's genetic diversity (h) across all 318 loci generated by all 20 PCs was maximum in RJF (0.3850) followed by in AS (0.1287). In WLH and RC, the h estimates were quite low (0.6669 and 0.0656, respectively). Similarly the means of Shannon's Information Index (I) across all 318 loci generated by all 20 PCs was maximum in RJF (0.5529), followed by AS (0.1889) and were quite low in WLH and RC (0.0993 and 0.0968, respectively). Mean Nei's genetic diversity (h) and mean Shannon's Information Index (I) across all 318 loci generated by all 20 PCs and across all populations were 0.2427 and 0.3910, respectively **(Table- 4.37)**.

4.2.2. Population Specific AFLP Markers

Some of the primer combinations were able to generate population specific bands **(Table 4.35)**. In RJF, a total of 26 bands population specific bands were found. In RJF, 13 out of 20 Primer combinations generated population specific bands and the number of population specific bands from these primer combinations was ranged from 1-5. In domestic chicken breeds very low number of population bands could be detected. 2 bands in RC and one band in AS were observed, while in WLH population no band was observed as population specific. Most of the population specific bands were present in very low frequency. The 14 out of 29 bands were present in less then 10 % allele frequency, all these bands were found in RJF. In RJF, allele frequencies of population-specific bands were ranged from 0.0253 (E01+T01 –01) to 0.3292 (E01+T03 -09). In RC, the allele frequencies of population-specific bands were ranged from 0.1181 (E03+T02 –05) to 0.1502 (E03+T06 -11). In AS, population-specific bands (E02+T03 -08) had the allele frequencies 0.4226, which was observed the highest for population-specific bands in any population.

4.2.3. Genetic differentiation and Gene flow

The G_{ST} values were estimated among the populations as per Nei's (1987) and the Nm was estimate using G_{ST} as per Nei's (1987). These estimates have been presented in **Table 4.36**. The G_{ST} estimates ranged from 0.2032 between RJF and AS populations to 0.3104 between WLH and RC populations. The G_{ST} values estimation

between RJF and domestic chicken populations namely as WLH, AS and RC was observed 0.2502, 0.2032 and 0.2723 respectively. Within domestic chicken groups, G_{ST} was observed 0.3104 (between WLH and RC) followed by 0.2342 (between WLH and AS) and 0.2077 (between AS and RC).

The gene flow estimates ranged from 1.1106 between WLH and RC populations to 1.9600 between RJF and AS populations. The gene flow estimation between RJF and domestic chicken populations namely as WLH, AS and RC was observed 1.4984, 1.9600, and 1.3362 respectively. When we compared gene flow between RJF and domestic chicken breeds, AS showed maximum gene flow with RJF. Within domestic chicken groups, the gene flow was observed 1.9069 (between AS and RC) followed by 1.6348 (between WLH and AS) and 1.1106 (between WLH and RC). Within domestic chicken breeds, RC showed maximum gene flow with AS. The G_{ST} and Nm value estimation across all loci and all populations were 0.3246 and 1.0405, respectively **(Table- 4.37)**.

4.2.4. Genetic distance and phylogenetic analysis

The genetic identity and distance were calculated for all possible population pairs as per Nei's (1972) and have been presented in **Table 4.38**. The genetic identity estimates ranged from 0.8017 between RJF and RC populations to 0.9442 between AS and RC populations. The genetic identity between the RJF and WLH populations was 0.8246, between the RJF and AS populations was 0.8377, and between the RJF and RC populations was 0.8017. Within domestic chicken breeds, the values for genetic identities were 0.9343 (between WLH and AS), 0.9361 (between WLH and RC), and 0.9442 (between AS and RC). In general, RJF showed more or less similar genetic identity (0.8017 to 0.8377) with all the domestic chicken breeds and estimates were comparatively lower than the estimates of genetic identity between domestic chicken breeds (0.9343 to 0.9442).

The genetic distances between the RJF and WLH populations was 0.1928, between the RJF and AS populations was 0.1771, and between the RJF and RC populations was 0.2210. The distances of all domestic chicken breeds from RJF showed little differences. The genetic distance between RJF and AS (Indian native chicken breed) populations was smaller than any other comparison. Within domestic chicken breeds Values for genetic distances were 0.0680 (between WLH and AS), 0.0660 (between WLH and RC), and 0.0575 (between AS and RC). Within domestic

chicken breeds, AS (Indian native chicken breed) showed smaller genetic distance with RC breed from any other population pair.

A phylogenetic tree of RJF and three domestic chicken breeds was constructed based on Nei's (1972) standard genetic distances using UPGMA method **(Fig 4.47-A)**. At about 91% genetic similarity, all four populations were divided in to two main clusters. The Wild RJF was quite distinct from domestic chicken breeds and formed a separate group (cluster-I) whereas the three domestic chicken breeds were clustered together in one group (cluster-II). Again at about 97% genetic similarity, domestic chicken breeds divided in to two sub clusters. WLH was placed in one sub-cluster and RC and AS were placed in another sub-cluster. These results indicated that all domestic chicken breeds showed closed relationships with each other. RC was found more close to AS (GI = 0.9442). As we compared the genetic relationships between RJF and domestic chicken breeds, AS (Indian native chicken breed) was found more close to the RJF (GI = 0.8377) than WLH (GI = 0.8246). To show the relationships among 76 individuals of all four chicken populations, a UPGMA dendrogram based on Nei's standard genetic distances was also constructed **(Fig 4.47- B)**. This dendrogram showed that within RJF population all individuals were placed distinctly from each other. In RJF, nine out of 20 RJF individuals formed 7 separate clusters and the remained 11 individuals form a separate cluster as a sub group with domestic chicken breeds.

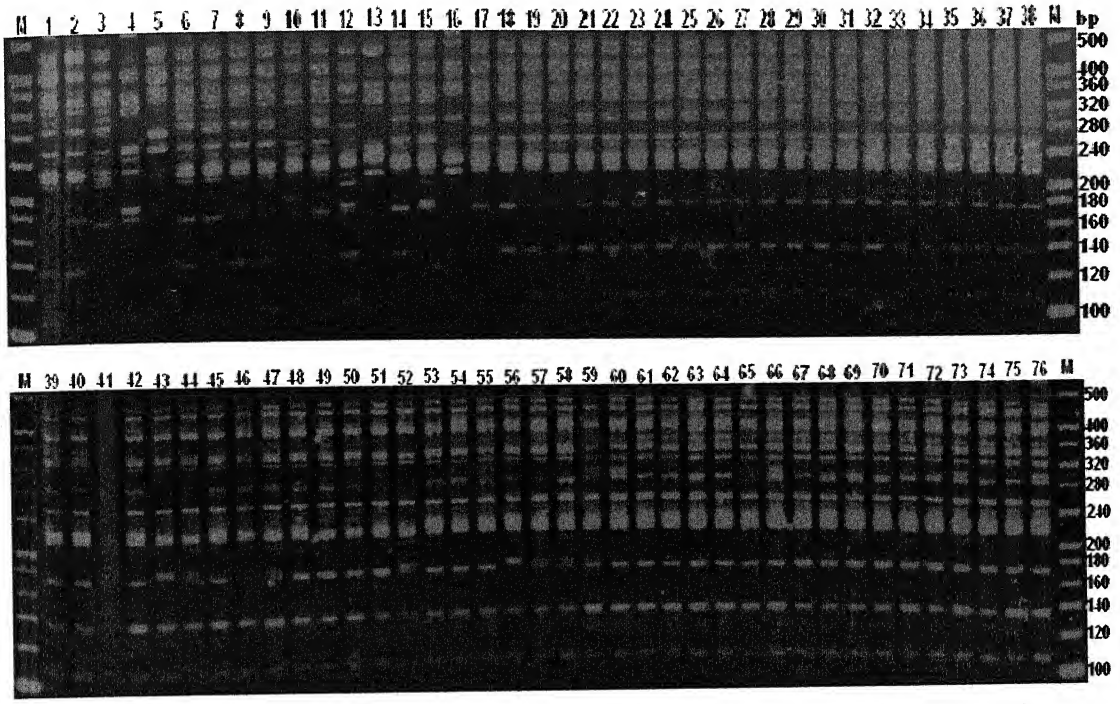


Fig 4.27. AFLP loci profile of different Chicken populations generated with Primer Combination **E04+T02**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

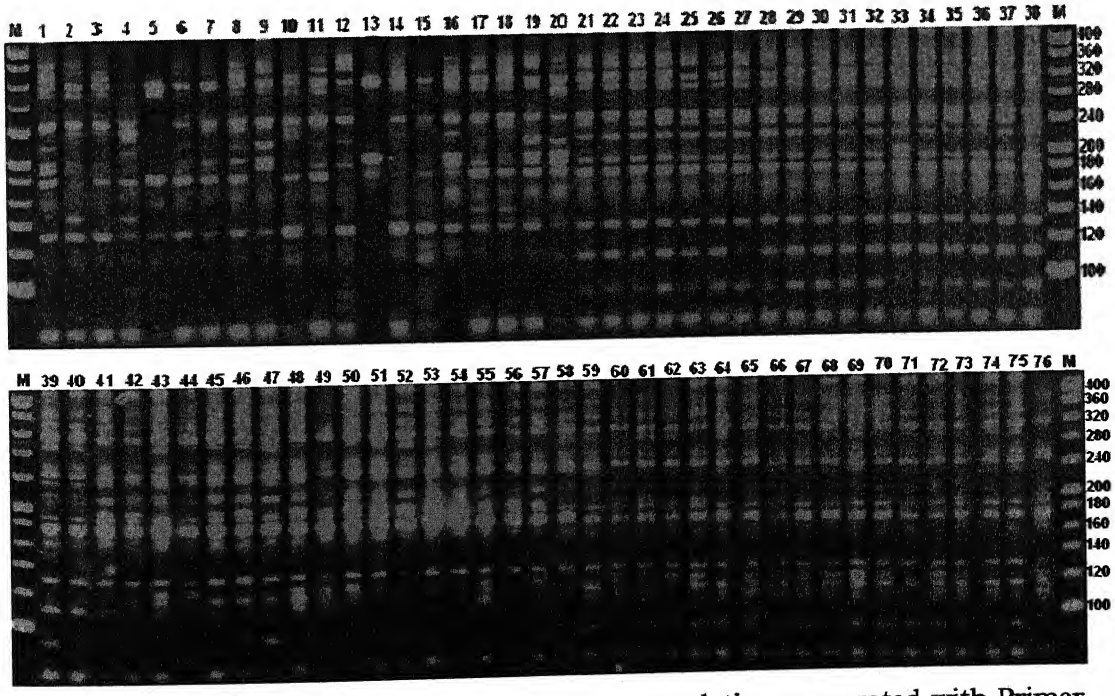


Fig 4.28. AFLP loci profile of different Chicken populations generated with Primer Combination **E03+T04**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

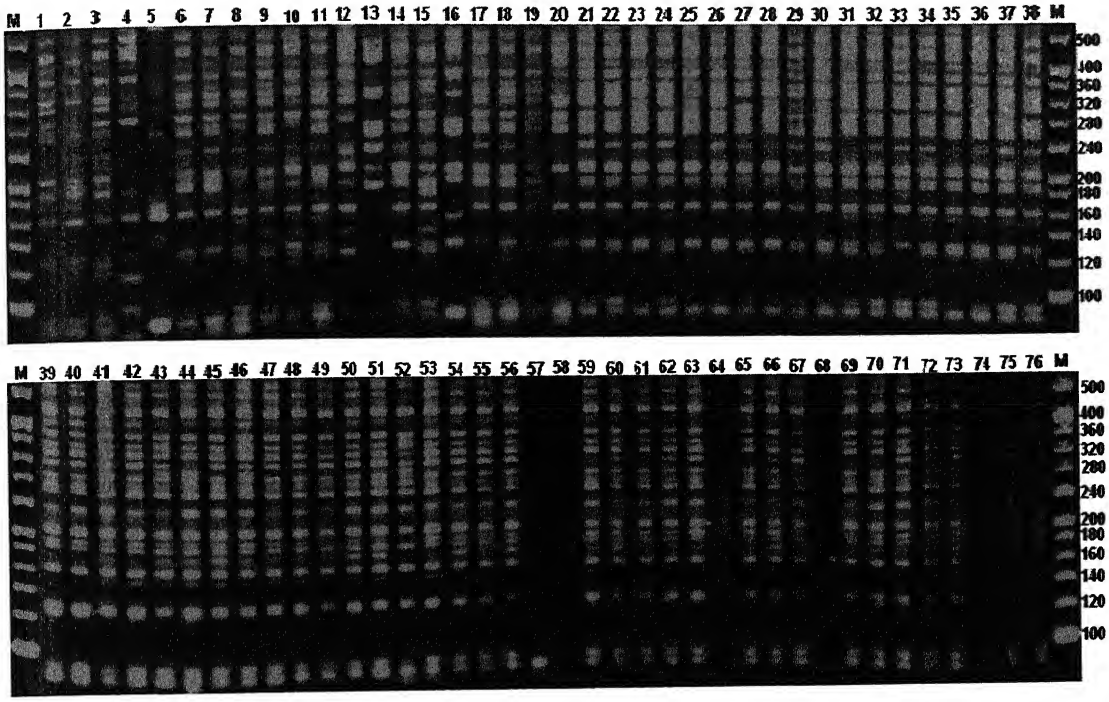


Fig 4.29. AFLP loci profile of different Chicken populations generated with Primer Combination **E03+T05**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

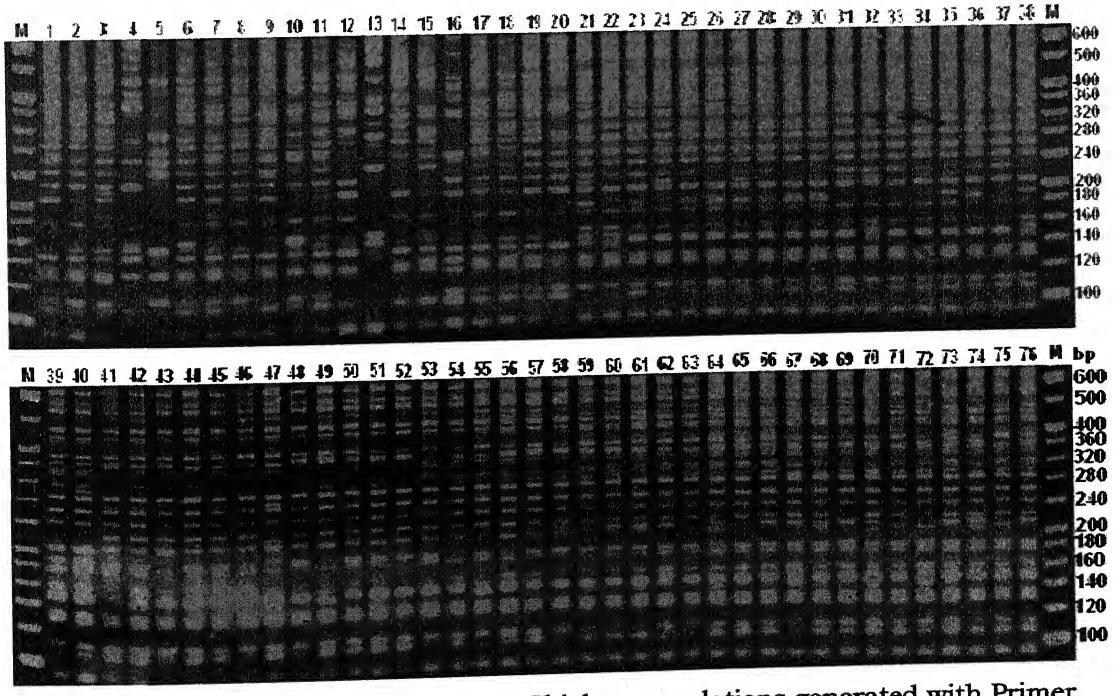


Fig 4.30. AFLP loci profile of different Chicken populations generated with Primer Combination **E04+T04**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

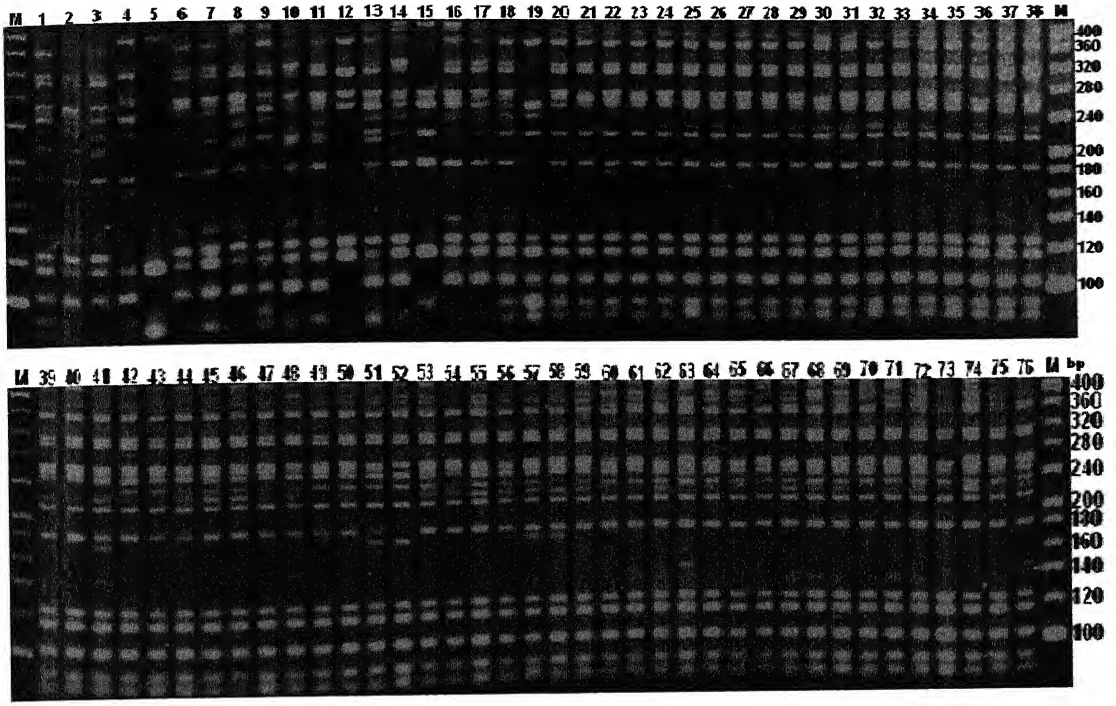


Fig 4.31. AFLP loci profile of different Chicken populations generated with Primer Combination **E02+T04**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

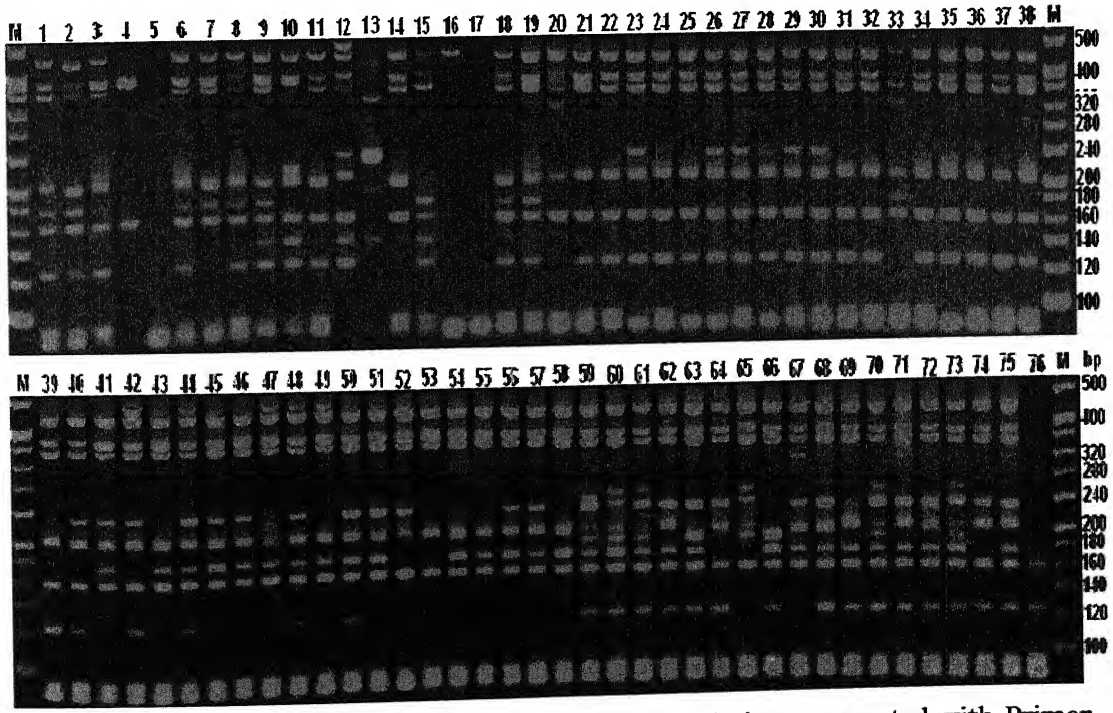


Fig 4.32. AFLP loci profile of different Chicken populations generated with Primer Combination **E03+T06**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

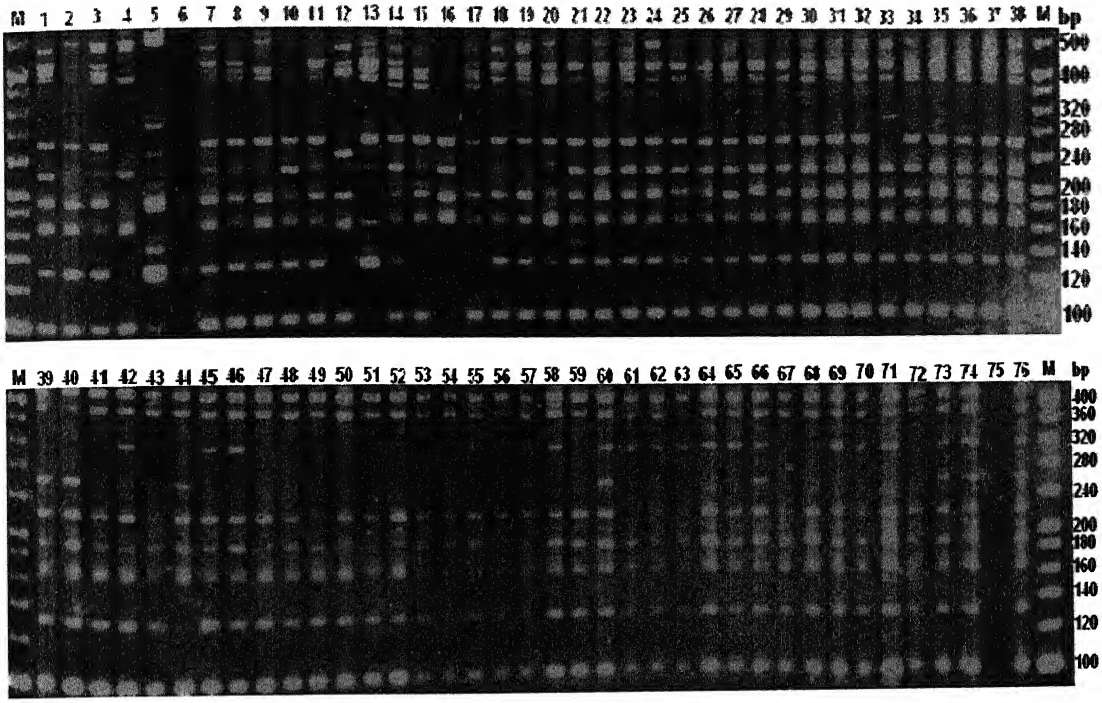


Fig 4.33. AFLP loci profile of different Chicken populations generated with Primer Combination **E04+T06**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

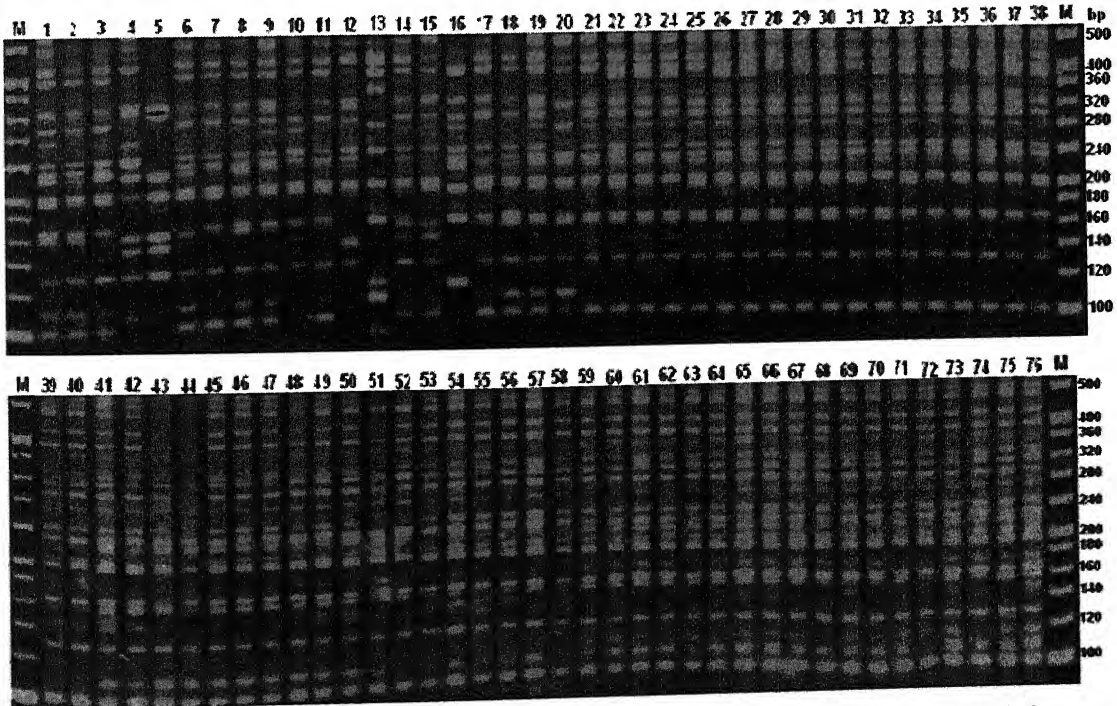


Fig 4.34. AFLP loci profile of different Chicken populations generated with Primer Combination **E02+T05**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

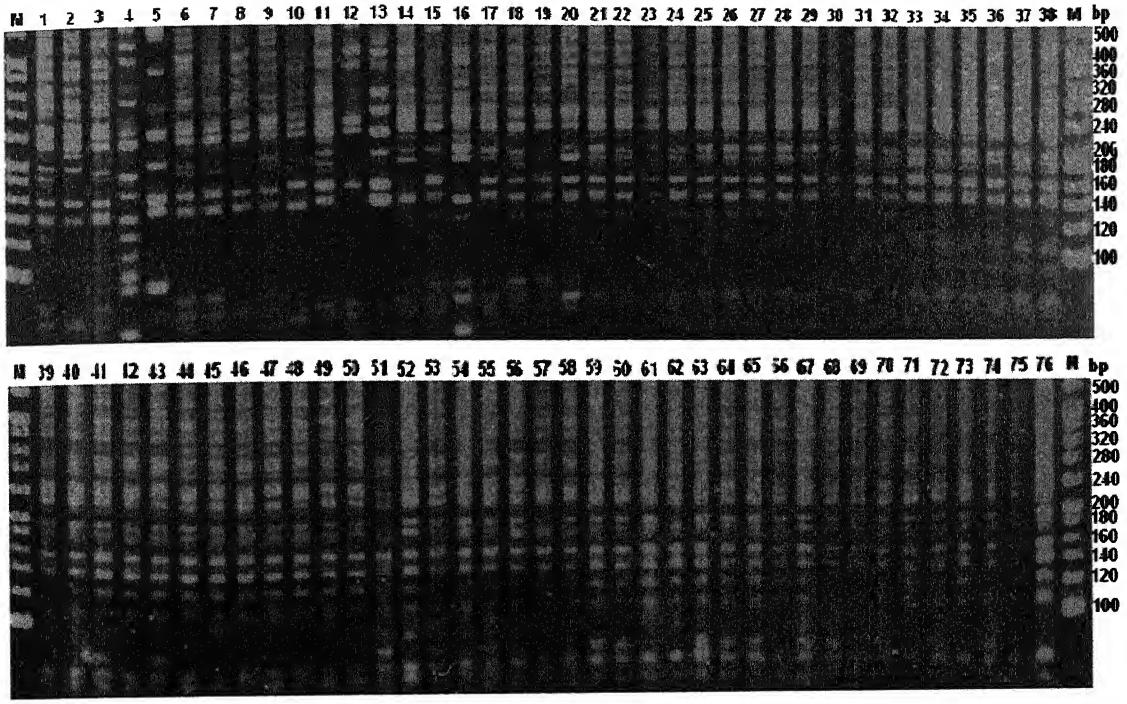


Fig 4.35. AFLP loci profile of different Chicken populations generated with Primer Combination **E04+T03**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

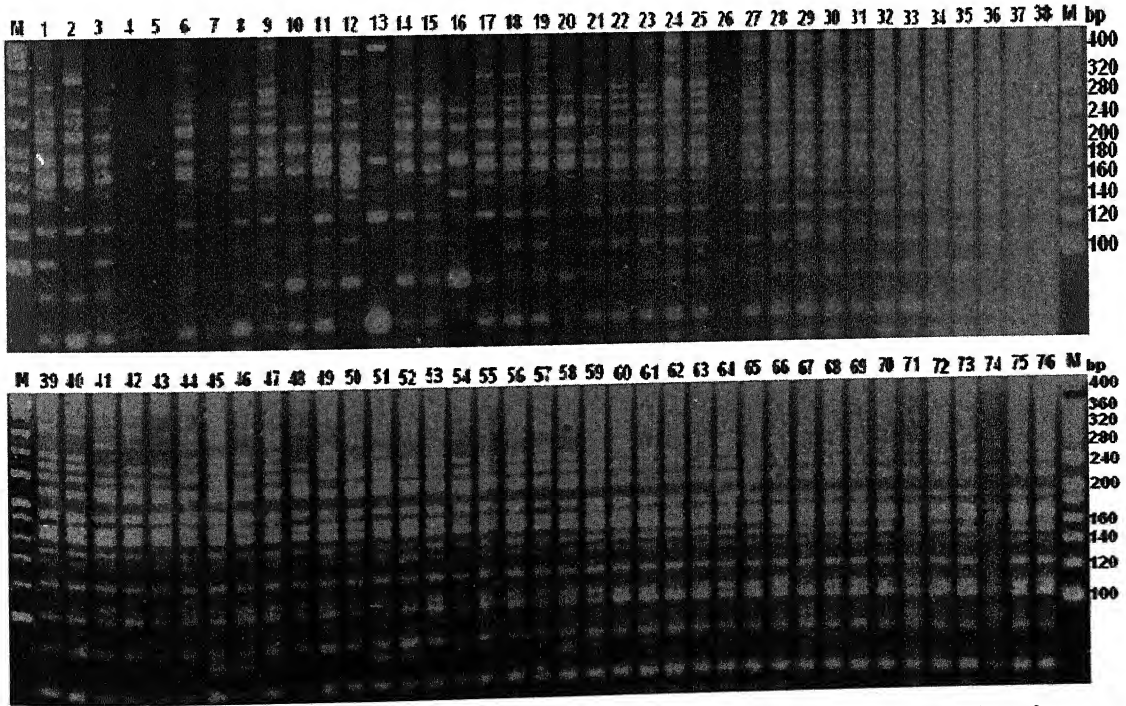


Fig 4.36. AFLP loci profile of different Chicken populations generated with Primer Combination **E02+T01**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

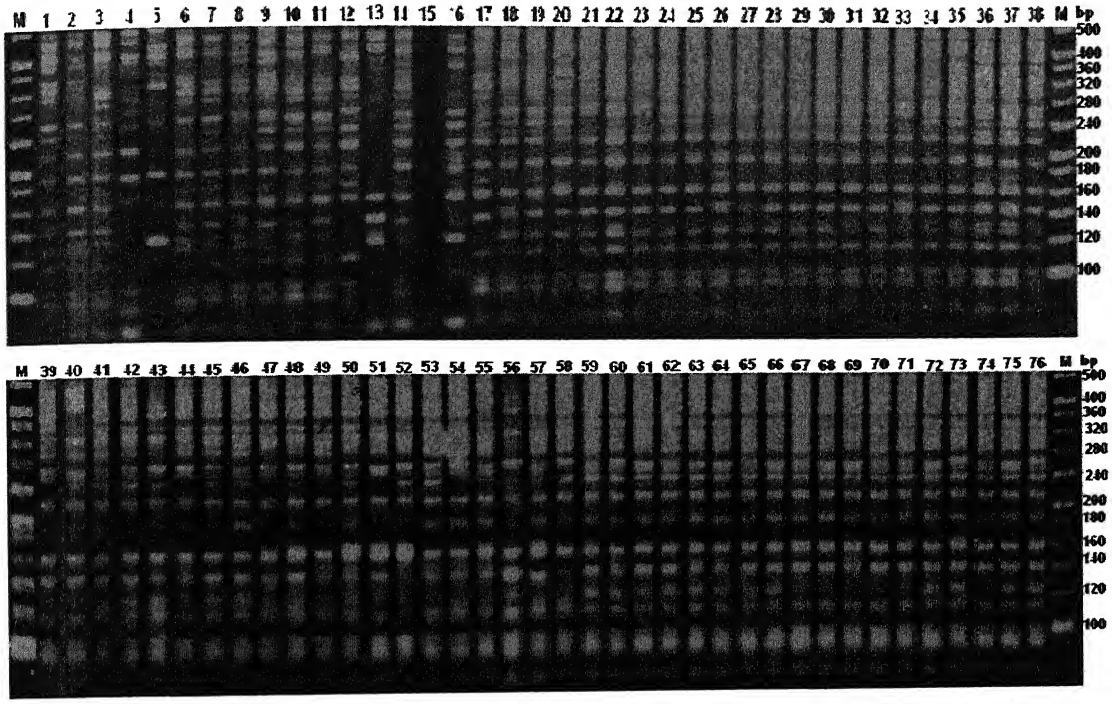


Fig 4.37. AFLP loci profile of different Chicken populations generated with Primer Combination **E04+T05**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

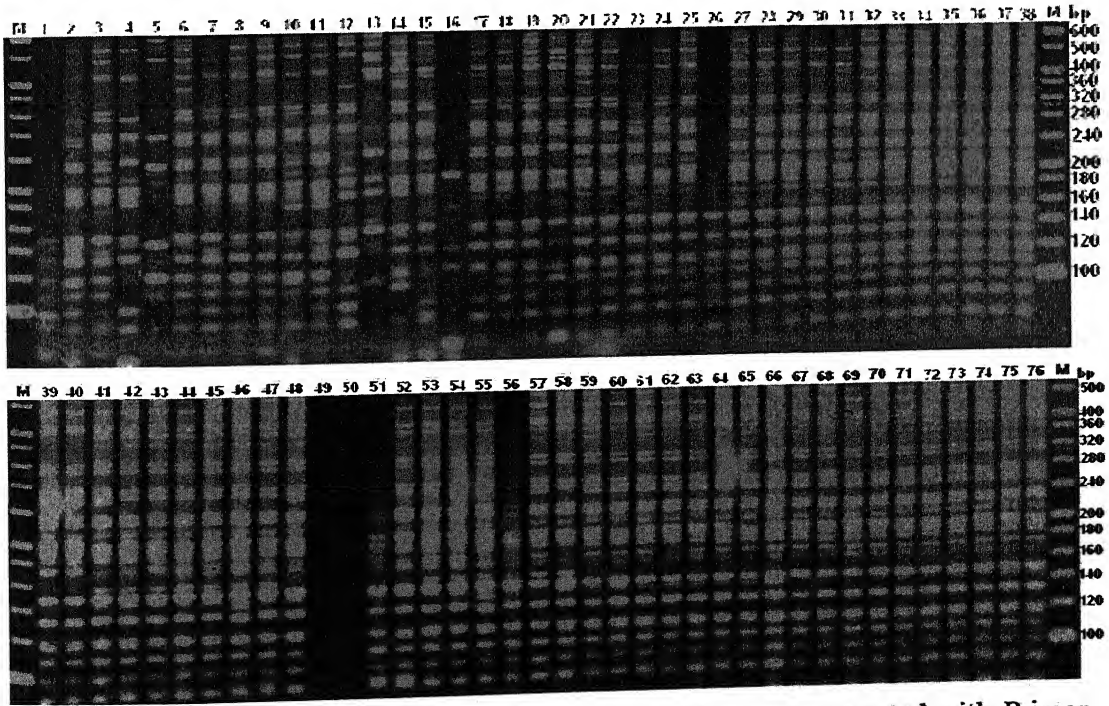


Fig 4.38. AFLP loci profile of different Chicken populations generated with Primer Combination **E03+T01**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

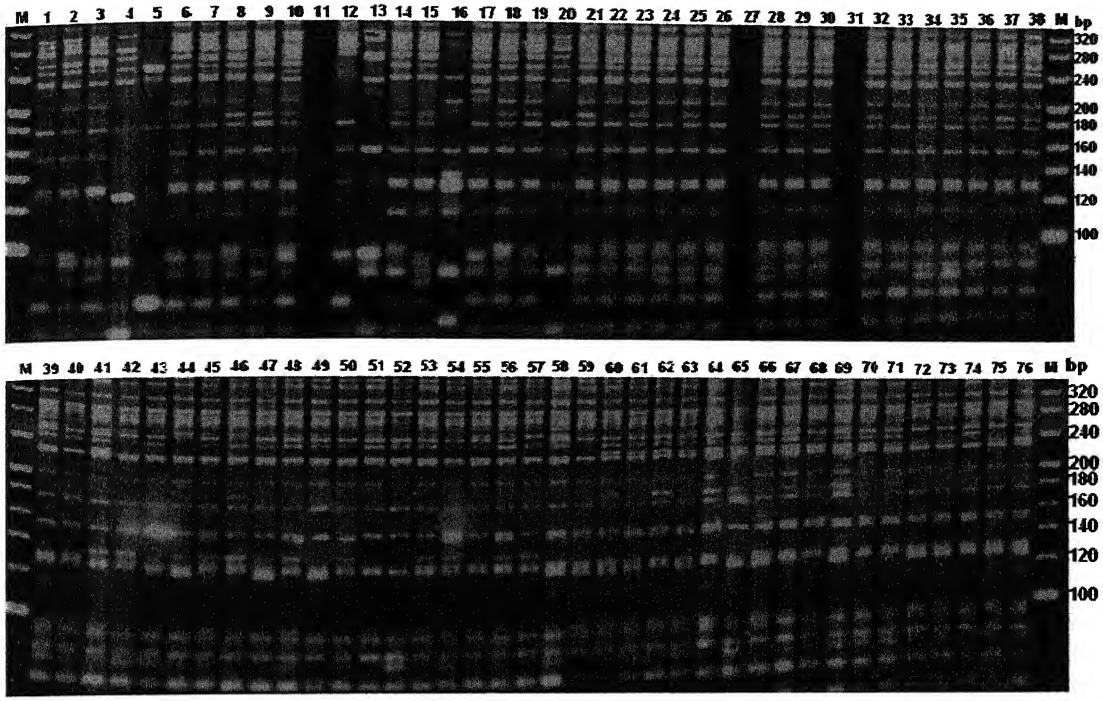


Fig 4.39. AFLP loci profile of different Chicken populations generated with Primer Combination **E02+T03**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

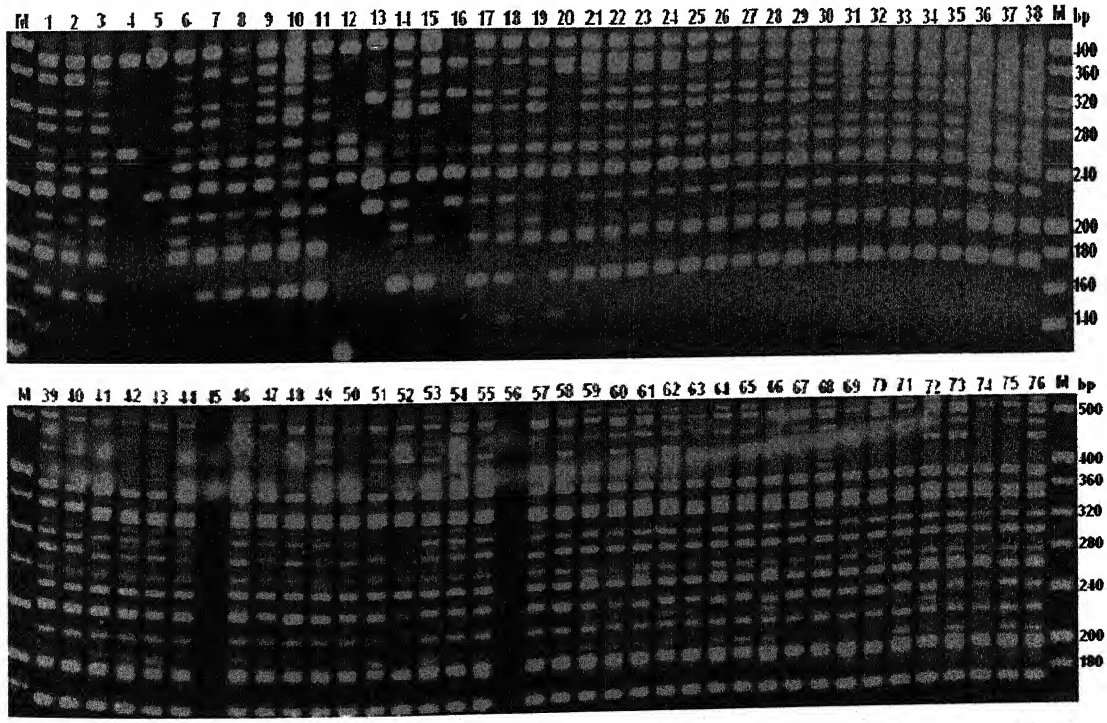


Fig 4.40. AFLP loci profile of different Chicken populations generated with Primer Combination **E03+T02**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

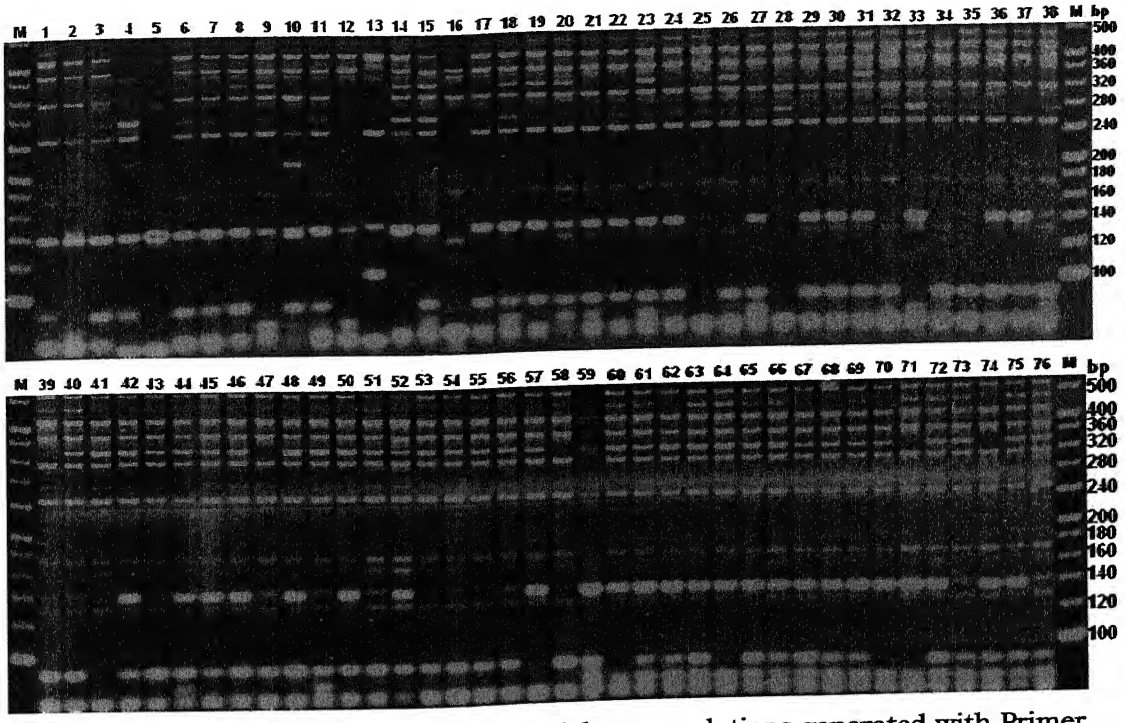


Fig 4.41. AFLP loci profile of different Chicken populations generated with Primer Combination **E01+T06**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

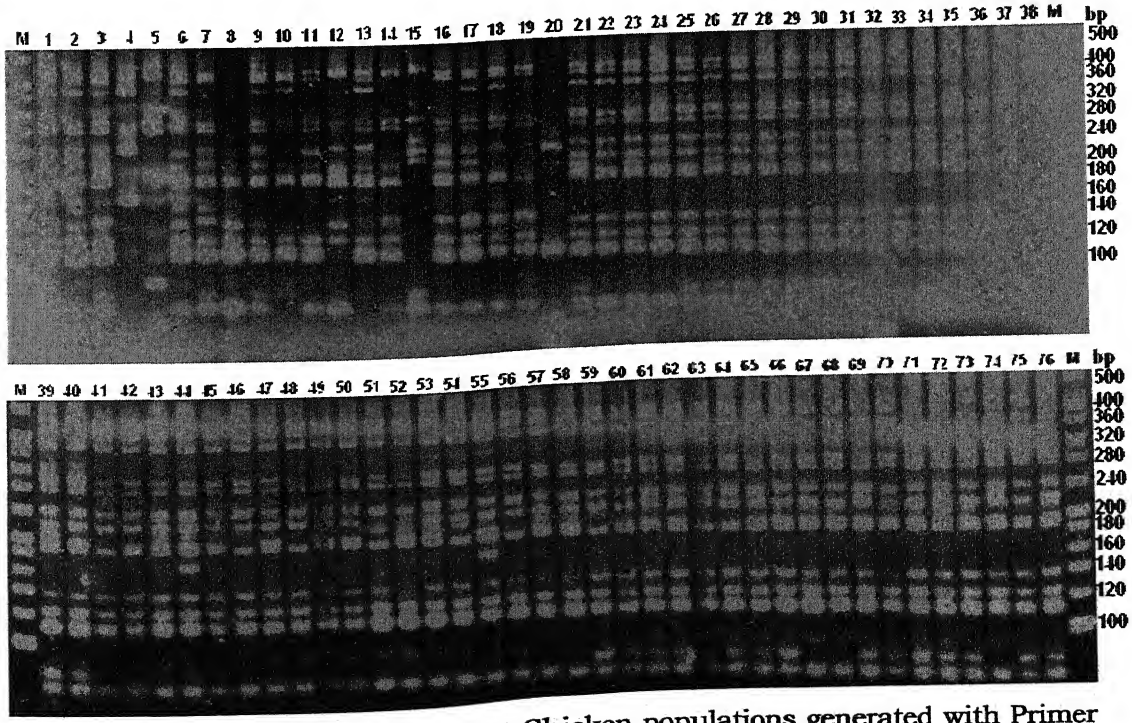


Fig 4.42. AFLP loci profile of different Chicken populations generated with Primer Combination **E01+T02**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

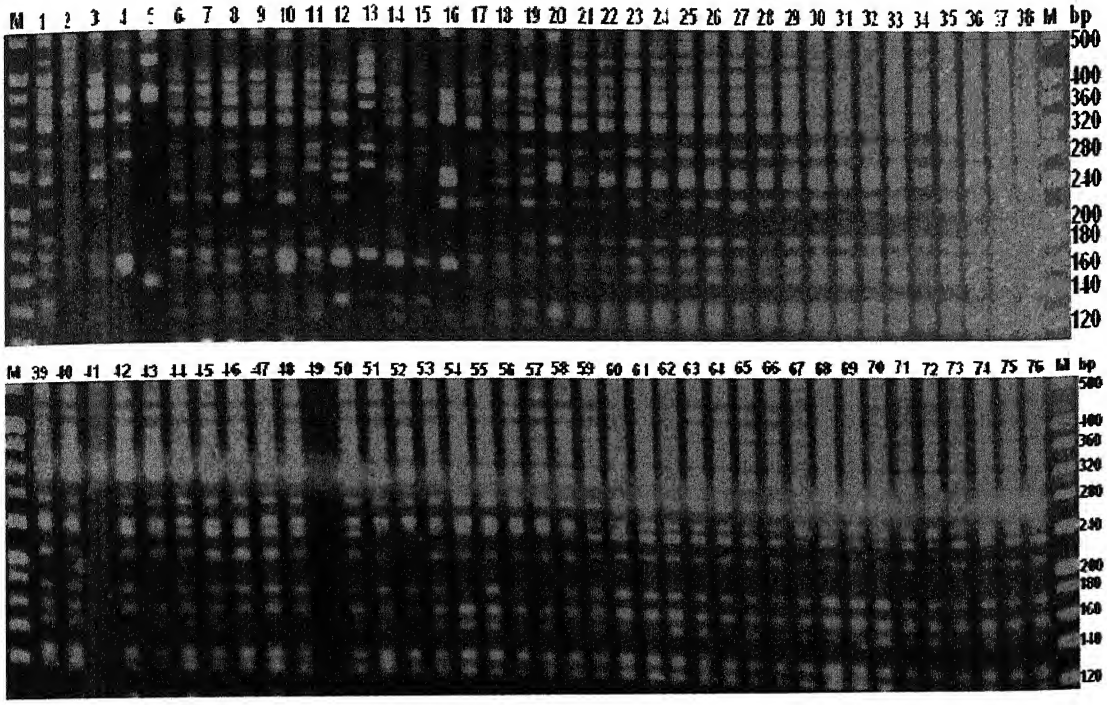


Fig 4.43. AFLP loci profile of different Chicken populations generated with Primer Combination **E01+T03**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

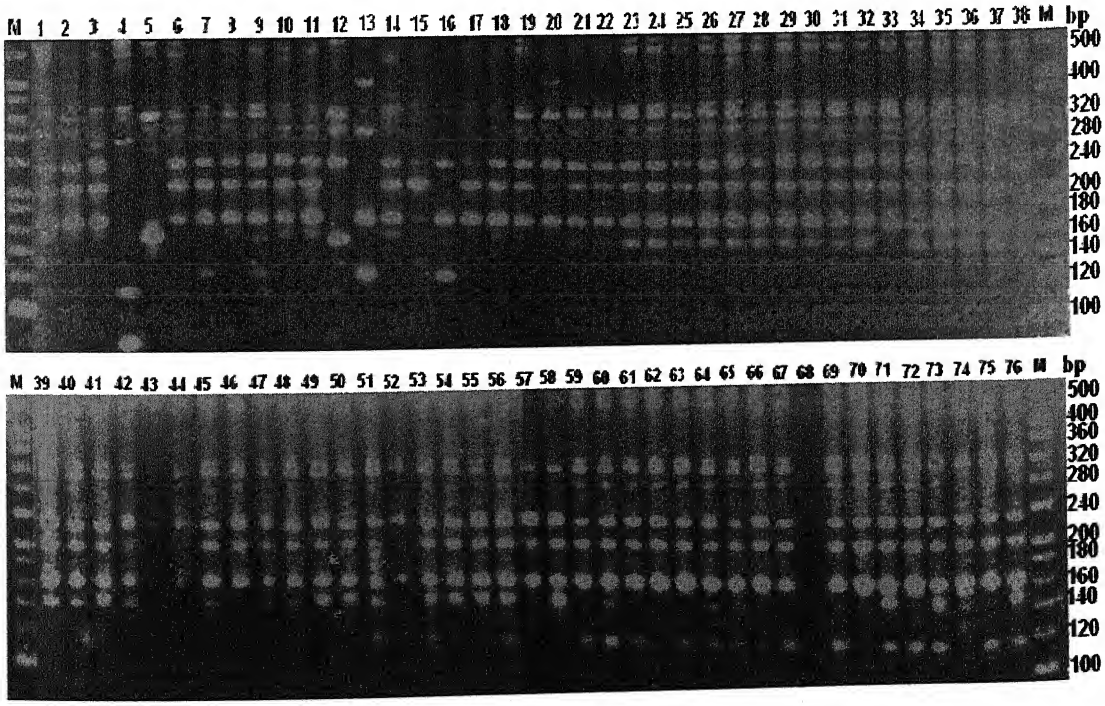


Fig 4.44. AFLP loci profile of different Chicken populations generated with Primer Combination **E02+T06**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

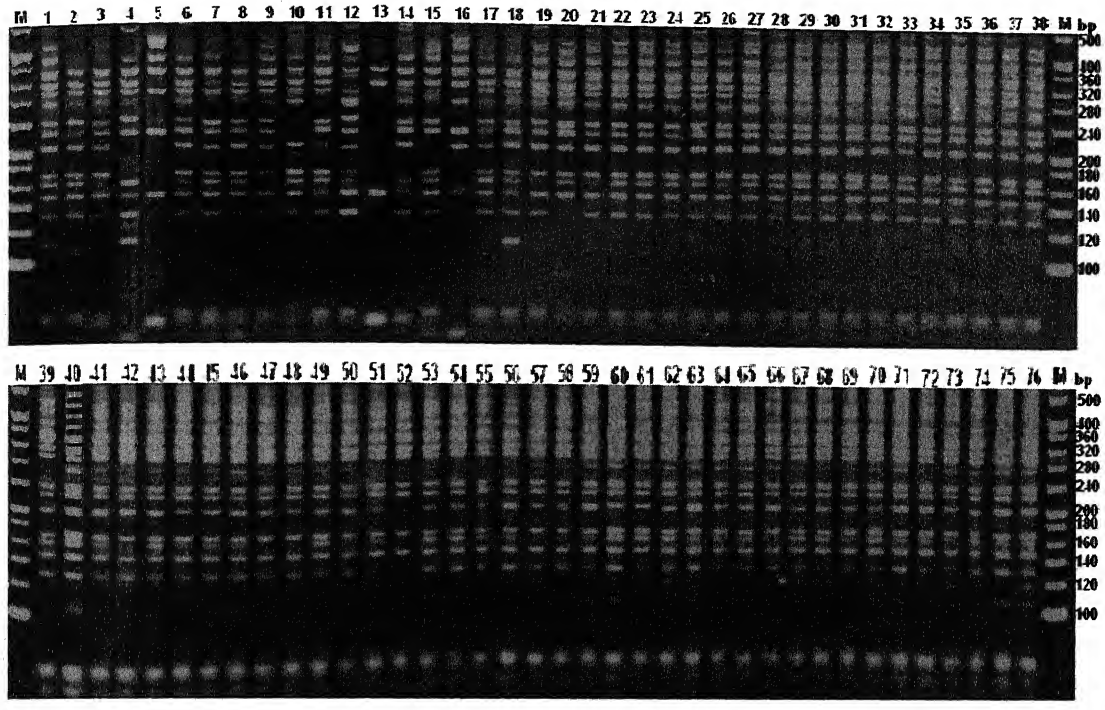


Fig 4.45. AFLP loci profile of different Chicken populations generated with Primer Combination **E02+T02**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

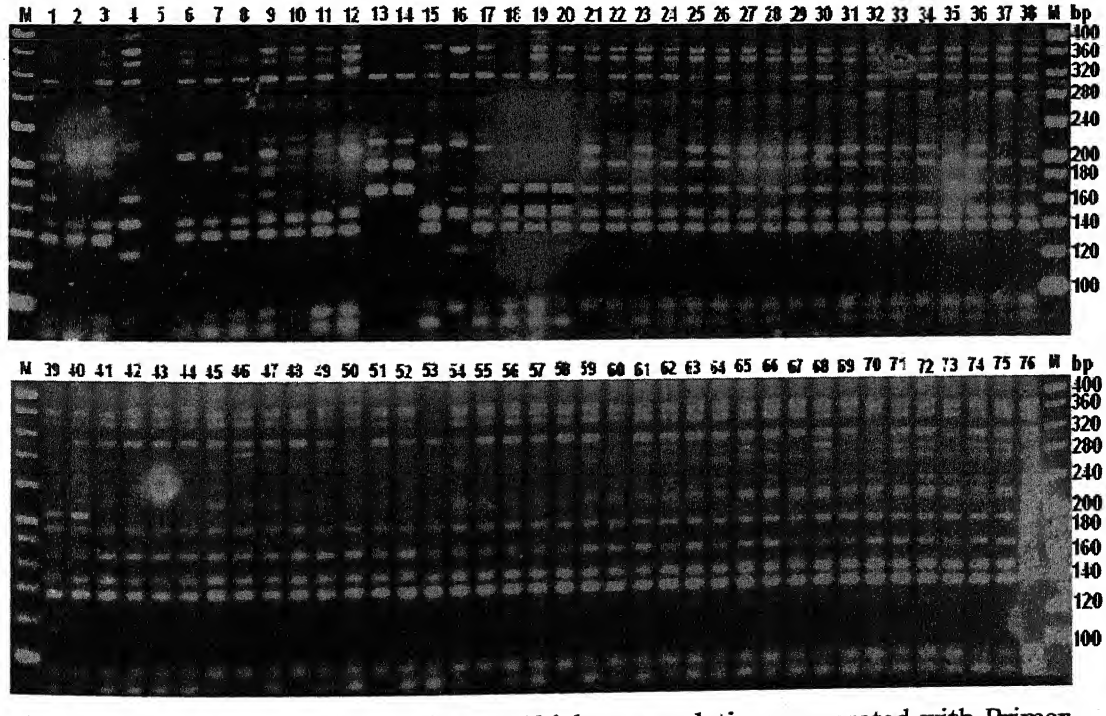


Fig 4.46. AFLP loci profile of different Chicken populations generated with Primer Combination **E03+T03**. Lane 1-20 : Red Jungle Fowl, 21-40 : White Leghorn, 41-58 : Aseel, 59-76 : Red Cornish. M : 20 bp DNA ladder

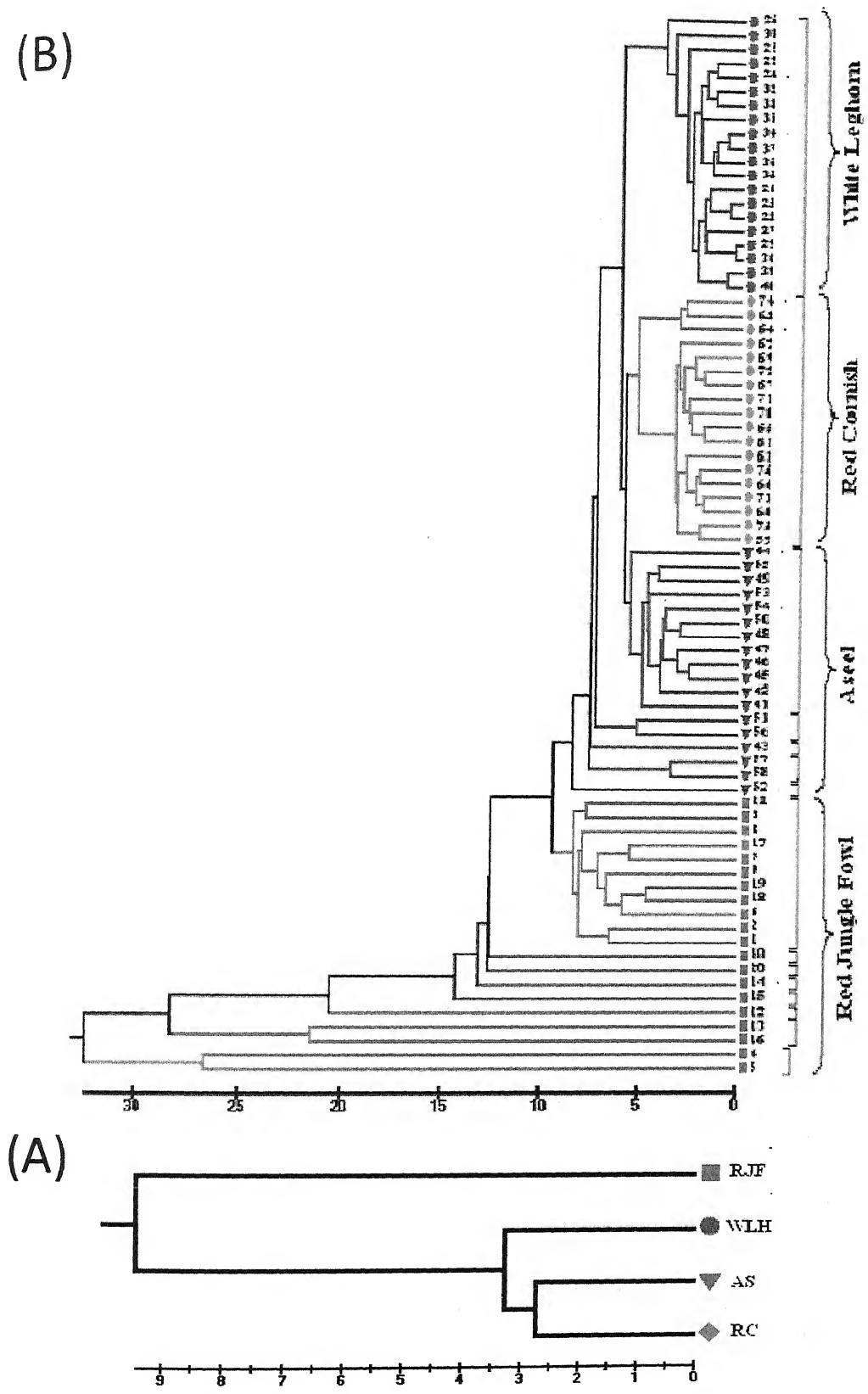


Fig. 4.47. UPGMA dendrogram based on Nei's (1972) standard genetic distance showing genetic relationships among **(A).** Red Jungle Fowl (RJF) and domestic chickens namely White Leghorn (WLH), Aseel (AS) and Red Cornish (RC) and **(B).** 76 individuals of wild and domestic chickens using AFLP markers

Table 4.32. Genetic Polymorphism parameters (size range, total number of bands N, polymorphic bands, % polymorphism) within Red jungle Fowl (RJF), White Leghorn (WL), Aseel (AS) and Red Cornish (RC) populations with 20 AFLP primer combinations

	Red Jungle Fowl				White Leghorn				Aseel				Red Cornish			
Primer Comb.	**Size range**	**N**	**Poly. bands**	**% poly.**	**Size range**	**N**	**Poly. bands**	**% poly.**	**Size range**	**N**	**Poly. bands**	**% poly.**	**Size range**	**N**	**Poly. bands**	**% poly.**
E01+T02	057-440	22	22	100	077-440	15	2	13	077-440	16	4	25	077-440	15	3	20
E01+T03	116-440	16	16	100	116-440	15	2	13	116-440	15	4	27	116-440	15	0	0
E01+T06	075-420	14	13	93	075-420	13	4	31	075-420	12	3	25	075-420	13	4	31
E02+T01	065-288	14	13	93	065-288	14	2	14	065-288	15	4	27	065-288	15	3	20
E02+T02	056-342	13	11	85	056-342	12	0	0	056-342	12	3	25	056-342	12	0	0
E02+T03	074-253	15	14	93	074-253	14	1	7	074-253	16	6	38	074-253	15	4	27
E02+T04	084-345	17	16	94	084-345	15	3	20	084-345	16	6	38	084-345	15	2	13
E02+T05	100-415	17	17	100	100-415	14	3	21	100-415	17	7	41	100-415	17	4	24
E02+T06	123-330	10	10	100	123-330	7	0	0	116-330	8	5	**63**	116-330	9	4	44
E03+T01	092-470	23	23	100	092-470	21	**11**	**52**	092-470	23	12	52	092-470	21	2	10
E03+T02	170-384	13	13	100	170-384	12	2	17	170-384	12	3	25	170-384	14	3	21
E03+T03	087-360	12	12	100	087-360	10	2	20	087-360	9	4	44	087-360	9	1	11
E03+T04	055-321	17	16	94	055-321	13	4	31	055-321	15	8	53	055-321	15	5	33
E03+T05	079-476	25	**25**	100	082-476	21	8	38	082-476	20	7	35	082-476	19	3	16
E03+T06	084-434	13	13	100	084-434	10	5	50	084-434	10	4	40	084-434	12	6	**50**
E04+T02	102-448	18	18	100	102-448	15	1	7	102-448	15	4	27	102-448	15	2	13
E04+T03	070-251	12	12	100	070-251	11	0	0	070-251	12	5	42	070-251	12	2	17
E04+T04	091-440	23	22	96	091-440	22	8	36	091-440	24	**14**	58	091-440	23	**8**	35
E04+T05	076-225	10	9	90	076-225	9	0	0	076-225	9	1	**11**	076-225	9	0	0
E04+T06	101-262	7	7	100	101-262	7	2	29	101-262	6	1	17	101-262	6	1	17
Sum		**311**	**302**			**270**	**60**			**282**	**105**			**281**	**57**	
Mean		**15.5**	**15.1**	**97 %**		**13.5**	**3**	**22 %**		**14.1**	**5.2**	**37 %**		**14.0**	**2.8**	**20 %**

Table 4.33. Nei's (1973) gene diversity (h) and Shannon's Information index (I) values at each locus for all 20 *Eco*R1/*Taq*1 primer combinations in different populations as well as across all the populations

AFLP	Size	Red Jungle Fowl		White Leghorn		Aseel		Red Cornish		Across all	
Loci	bps	h	I	h	I	h	I	h	I	h	I
E01+T02-01	057	0.0494	0.1181	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0132	0.0400
E01+T02-02	061	0.1439	0.2740	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0402	0.1001
E01+T02-03	077	**0.5000**	**0.6931**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2285	0.3894
E01+T02-04	085	0.2321	0.3939	**0.3125**	**0.4916**	0.0000	0.0000	**0.4984**	**0.6915**	0.3335	0.5159
E01+T02-05	093	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.3796	0.5674	0.4283	0.6196
E01+T02-06	115	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E01+T02-07	128	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E01+T02-08	140	0.2321	0.3939	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3519	0.5367
E01+T02-09	149	0.3492	0.5337	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1116	0.2251
E01+T02-10	162	0.1889	0.3372	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0540	0.1269
E01+T02-11	169	0.1439	0.2740	0.0000	0.0000	0.1078	0.2192	0.0000	0.0000	0.0658	0.1487
E01+T02-12	186	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E01+T02-13	197	0.4944	0.6876	0.0000	0.0000	**0.4880**	**0.6811**	0.4832	0.6762	**0.4557**	**0.6481**
E01+T02-14	209	0.1439	0.2740	0.3125	0.4916	0.3412	0.5247	0.0000	0.0000	0.2161	0.3733
E01+T02-15	223	0.4954	0.6886	0.0000	0.0000	0.4444	0.6365	0.0000	0.0000	0.3466	0.5308
E01+T02-16	250	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E01+T02-17	260	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0266	0.0715
E01+T02-18	284	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E01+T02-19	297	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E01+T02-20	375	0.4416	0.6336	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2907	0.4661
E01+T02-21	409	0.4832	0.6763	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2629	0.4324
E01+T02-22	440	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E01+T03-01	116	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2125	0.3687
E01+T03-02	125	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E01+T03-03	144	0.4832	0.6763	0.0000	0.0000	0.4747	0.6677	0.0000	0.0000	0.4137	0.6042
E01+T03-04	160	0.3472	0.5314	0.0000	0.0000	0.4571	0.6496	0.0000	0.0000	0.2363	0.3993
E01+T03-05	175	0.4832	0.6763	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2687	0.4395
E01+T03-06	212	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2337	0.3960
E01+T03-07	235	0.4954	0.6886	0.4954	0.6886	0.4479	0.6401	0.0000	0.0000	**0.4926**	**0.6857**
E01+T03-08	252	0.4746	0.6675	**0.5000**	**0.6931**	0.0000	0.0000	0.0000	0.0000	0.3646	0.5509
E01+T03-09	265	0.4416	0.6336	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1621	0.3001
E01+T03-10	276	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2337	0.3960
E01+T03-11	310	0.4954	0.6886	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2522	0.4193
E01+T03-12	325	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E01+T03-13	343	**0.5000**	**0.6931**	0.0000	0.0000	**0.4910**	**0.6841**	0.0000	0.0000	0.3529	0.5378
E01+T03-14	374	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E01+T03-15	400	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2337	0.3960
E01+T03-16	440	0.1439	0.2740	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3742	0.5614
E01+T06-01	075	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E01+T06-02	081	0.3492	0.5337	0.1889	0.3372	0.1078	0.2192	0.0000	0.0000	0.4472	0.6394
E01+T06-03	090	**0.4954**	**0.6886**	**0.4746**	**0.6675**	**0.4444**	**0.6365**	**0.4984**	**0.6915**	**0.4920**	**0.6851**
E01+T06-04	133	0.3832	0.5714	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3141	0.4936
E01+T06-05	142	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E01+T06-06	172	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.3603	0.5461	0.2394	0.4032
E01+T06-07	217	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0266	0.0715
E01+T06-08	252	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E01+T06-09	274	0.4832	0.6763	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2629	0.4324
E01+T06-10	303	0.4325	0.6240	0.4325	0.6240	0.0000	0.0000	0.1591	0.2958	0.4714	0.6643
E01+T06-11	316	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E01+T06-12	345	0.3492	0.5337	0.1439	0.2740	0.4444	0.6365	0.4444	0.6365	0.4782	0.6712
E01+T06-13	382	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E01+T06-14	420	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293

Continued....

E02+T01-1	065	0.3536	0.5386	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1109	0.2239
E02+T01-2	073	0.0000	0.0000	0.0000	0.0000	0.3796	0.5674	0.1591	0.2958	0.1524	0.2864
E02+T01-3	084	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E02+T01-4	102	0.3983	0.5877	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3031	0.4808
E02+T01-5	108	0.3263	0.5076	0.2859	0.4603	**0.4832**	**0.6762**	**0.4984**	**0.6915**	**0.4660**	**0.6587**
E02+T01-6	125	0.3536	0.5386	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1109	0.2239
E02+T01-7	142	0.3263	0.5076	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3248	0.5060
E02+T01-8	152	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1527	0.2868
E02+T01-9	170	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1832	0.3295
E02+T01-10	178	**0.4966**	**0.6898**	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2889	0.4639
E02+T01-11	193	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1832	0.3295
E02+T01-12	222	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1832	0.3295
E02+T01-13	244	0.4923	0.6855	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2469	0.4127
E02+T01-14	265	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1527	0.2868
E02+T01-15	288	0.2431	0.4079	**0.4923**	**0.6855**	0.3796	0.5674	0.4880	0.6811	0.4305	0.6219
E02+T02-01	056	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T02-02	065	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E02+T02-03	108	0.1439	0.2740	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0402	0.1001
E02+T02-04	132	0.4944	0.6876	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2868	0.4614
E02+T02-05	150	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E02+T02-06	164	0.4746	0.6675	0.0000	0.0000	**0.4880**	**0.6811**	0.0000	0.0000	**0.3634**	**0.5495**
E02+T02-07	175	**0.5000**	**0.6931**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2285	0.3894
E02+T02-08	206	0.4944	0.6876	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2868	0.4614
E02+T02-09	226	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E02+T02-10	241	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E02+T02-11	299	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E02+T02-12	316	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E02+T02-13	342	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E02+T03-1	074	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1855	0.3325
E02+T03-2	080	0.1511	0.2844	0.0000	0.0000	0.4142	0.6047	**0.4984**	**0.6915**	**0.4983**	**0.6914**
E02+T03-3	086	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1546	0.2895
E02+T03-4	094	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1855	0.3325
E02+T03-5	114	**0.4966**	**0.6898**	0.0000	0.0000	**0.4880**	**0.6811**	0.0000	0.0000	0.3865	0.5749
E02+T03-6	125	0.4966	0.6898	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2103	0.3658
E02+T03-7	132	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1855	0.3325
E02+T03-8	142	0.0000	0.0000	0.0000	0.0000	0.4880	0.6811	0.0000	0.0000	0.1867	0.3342
E02+T03-9	154	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1546	0.2895
E02+T03-10	180	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1546	0.2895
E02+T03-11	187	0.4771	0.6701	0.4880	0.6811	0.1078	0.2192	0.4694	0.6623	0.4304	0.6218
E02+T03-12	202	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1855	0.3325
E02+T03-13	230	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E02+T03-14	236	0.0519	0.1230	0.0000	0.0000	0.0548	0.1283	0.4444	0.6365	0.2930	0.4688
E02+T03-15	246	0.4789	0.6719	0.0000	0.0000	0.3603	0.5461	0.4984	0.6915	0.4012	0.5908
E02+T03-16	253	0.4771	0.6701	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2660	0.4363
E02+T04-01	084	**0.4954**	**0.6886**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2467	0.4124
E02+T04-02	090	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2775	0.4502
E02+T04-03	101	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T04-04	117	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E02+T04-05	126	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T04-06	168	0.0974	0.2024	0.0494	0.1181	0.2552	0.4230	0.0000	0.0000	0.1053	0.2151
E02+T04-07	181	0.4746	0.6675	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2657	0.4359
E02+T04-08	204	0.2321	0.3939	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0680	0.1526
E02+T04-09	212	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T04-10	220	0.1889	0.3372	0.3472	0.5314	**0.4832**	**0.6762**	**0.4444**	**0.6365**	0.4952	0.6884
E02+T04-11	229	0.4954	0.6886	0.0000	0.0000	0.4444	0.6365	0.0000	0.0000	**0.4996**	**0.6928**
E02+T04-12	242	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E02+T04-13	251	0.4954	0.6886	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.3200	0.5003
E02+T04-14	258	0.3472	0.5314	**0.4746**	**0.6675**	0.4444	0.6365	0.4444	0.6365	0.4790	0.6720
E02+T04-15	290	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623

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E02+T04-16	302	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E02+T04-17	345	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E02+T05-01	100	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2285	0.3894
E02+T05-02	111	0.3472	0.5314	0.0000	0.0000	0.3603	0.5461	0.3603	0.5461	**0.4912**	**0.6843**
E02+T05-03	123	0.1889	0.3372	0.0000	0.0000	0.2083	0.3631	**0.4985**	**0.6917**	0.2792	0.4523
E02+T05-04	132	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E02+T05-05	140	0.3832	0.5714	0.4325	0.6240	**0.4694**	**0.6623**	0.2083	0.3631	0.4636	0.6563
E02+T05-06	146	0.3125	0.4916	0.0000	0.0000	0.3796	0.5674	0.1591	0.2958	0.2291	0.3901
E02+T05-07	159	0.4416	0.6336	**0.4832**	**0.6763**	0.0000	0.0000	0.0000	0.0000	0.4437	0.6357
E02+T05-08	165	**0.5000**	**0.6931**	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.3046	0.4824
E02+T05-09	224	0.4416	0.6336	0.4325	0.6240	0.3603	0.5461	0.0000	0.0000	0.4320	0.6235
E02+T05-10	233	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T05-11	244	0.4325	0.6240	0.0000	0.0000	0.4444	0.6365	0.0000	0.0000	0.2717	0.4432
E02+T05-12	278	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2775	0.4502
E02+T05-13	296	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T05-14	317	0.3492	0.5337	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3246	0.5057
E02+T05-15	331	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E02+T05-16	391	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E02+T05-17	415	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E02+T06-01	116	0.0000	0.0000	0.0000	0.0000	0.1679	0.3083	**0.4996**	**0.6927**	0.2401	0.4041
E02+T06-02	123	0.1889	0.3372	0.0000	0.0000	0.0000	0.0000	0.0579	0.1342	0.0683	0.1530
E02+T06-03	142	0.1889	0.3372	0.0000	0.0000	0.4872	0.6803	0.3964	0.5856	**0.4999**	**0.6931**
E02+T06-04	152	0.2321	0.3939	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0698	0.1557
E02+T06-05	165	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E02+T06-06	200	**0.4954**	**0.6886**	0.0000	0.0000	0.3674	0.5540	0.0000	0.0000	0.3245	0.5056
E02+T06-07	225	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E02+T06-08	277	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0274	0.0731
E02+T06-09	288	0.3472	0.5314	0.0000	0.0000	0.3964	0.5856	0.0000	0.0000	0.3516	0.5363
E02+T06-10	312	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E02+T06-11	330	0.4954	0.6886	0.0000	0.0000	**0.4996**	**0.6927**	0.3674	0.5540	0.4317	0.6232
E03+T01-01	092	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1563	0.2919
E03+T01-02	102	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2125	0.3687
E03+T01-03	111	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1563	0.2919
E03+T01-04	122	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1136	0.2282
E03+T01-05	132	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1563	0.2919
E03+T01-06	138	0.2321	0.3939	0.0000	0.0000	0.1208	0.2394	0.0000	0.0000	0.0953	0.1990
E03+T01-07	144	0.3492	0.5337	0.0000	0.0000	0.4747	0.6677	**0.4984**	**0.6915**	0.3972	0.5865
E03+T01-08	153	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1136	0.2282
E03+T01-09	160	0.0494	0.1181	**0.4954**	**0.6886**	**0.4910**	**0.6841**	0.0000	0.0000	0.3767	0.5642
E03+T01-10	172	0.3125	0.4916	0.0000	0.0000	0.5000	0.6931	0.0000	0.0000	0.4395	0.6313
E03+T01-11	181	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1563	0.2919
E03+T01-12	192	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1136	0.2282
E03+T01-13	202	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1563	0.2919
E03+T01-14	213	0.4832	0.6763	0.4325	0.6240	0.4910	0.6841	0.4444	0.6365	0.4872	0.6803
E03+T01-15	229	**0.4954**	**0.6886**	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.3300	0.5119
E03+T01-16	237	0.4944	0.6876	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.2969	0.4734
E03+T01-17	262	0.3125	0.4916	0.4944	0.6876	0.2321	0.3939	0.0000	0.0000	**0.4986**	**0.6918**
E03+T01-18	274	0.4954	0.6886	0.3472	0.5314	0.4571	0.6496	0.0000	0.0000	0.4075	0.5975
E03+T01-19	284	0.4746	0.6675	0.3472	0.5314	0.4571	0.6496	0.0000	0.0000	0.3664	0.5529
E03+T01-20	315	0.4944	0.6876	0.3472	0.5314	0.4571	0.6496	0.0000	0.0000	0.3826	0.5707
E03+T01-21	325	0.4954	0.6886	0.3472	0.5314	0.4571	0.6496	0.0000	0.0000	0.4075	0.5975
E03+T01-22	418	0.4944	0.6876	0.3472	0.5314	0.4571	0.6496	0.0000	0.0000	0.3826	0.5707
E03+T01-23	470	0.4746	0.6675	0.3472	0.5314	0.4571	0.6496	0.0000	0.0000	0.3664	0.5529
E03+T02-01	170	0.4954	0.6886	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2522	0.4193
E03+T02-02	198	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2337	0.3960
E03+T02-03	206	0.4416	0.6336	**0.5000**	**0.6931**	**0.4930**	**0.6862**	**0.4880**	**0.6811**	**0.4879**	**0.6810**
E03+T02-04	222	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352

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E03+T02-05	230	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2083	0.3631	0.0558	0.1303
E03+T02-06	242	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1136	0.2282
E03+T02-07	265	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1874	0.3352
E03+T02-08	280	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2125	0.3687
E03+T02-09	302	0.4954	0.6886	0.0000	0.0000	0.3750	0.5623	0.0000	0.0000	0.3225	0.5033
E03+T02-10	311	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0548	0.1283	0.0406	0.1008
E03+T02-11	322	**0.5000**	**0.6931**	0.0000	0.0000	0.3750	0.5623	0.0000	0.0000	0.3068	0.4850
E03+T02-12	347	0.2733	0.4452	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.4788	0.6718
E03+T02-13	356	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2337	0.3960
E03+T02-14	384	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1136	0.2282
E03+T03-1	087	**0.4997**	**0.6928**	**0.5000**	**0.6931**	**0.4985**	**0.6917**	0.0000	0.0000	**0.4757**	**0.6687**
E03+T03-2	095	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1509	0.2841
E03+T03-3	126	0.3983	0.5877	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1294	0.2525
E03+T03-4	136	0.4966	0.6898	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2055	0.3594
E03+T03-5	149	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1509	0.2841
E03+T03-6	168	0.4923	0.6855	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2442	0.4092
E03+T03-7	192	0.4923	0.6855	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2442	0.4092
E03+T03-8	210	0.4923	0.6855	0.5000	0.6931	0.0000	0.0000	0.0000	0.0000	0.3692	0.5560
E03+T03-9	218	0.1511	0.2844	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0408	0.1014
E03+T03-10	308	0.3536	0.5386	0.0000	0.0000	0.3603	0.5461	**0.3603**	**0.5461**	0.2839	0.4579
E03+T03-11	340	0.4384	0.6302	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2390	0.4027
E03+T03-12	360	0.3536	0.5386	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2031	0.3562
E03+T04-1	055	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E03+T04-2	074	0.4746	0.6675	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2657	0.4359
E03+T04-3	082	0.1439	0.2740	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0402	0.1001
E03+T04-4	091	0.2321	0.3939	**0.3472**	**0.5314**	0.2997	0.4767	0.0000	0.0000	**0.4992**	**0.6924**
E03+T04-5	108	0.1889	0.3372	0.3472	0.5314	0.4142	0.6047	0.0000	0.0000	0.4971	0.6902
E03+T04-6	115	0.3832	0.5714	0.0000	0.0000	0.4832	0.6762	0.0000	0.0000	0.4939	0.6871
E03+T04-7	121	0.4416	0.6336	0.0000	0.0000	0.3796	0.5674	**0.4880**	**0.6811**	0.3720	0.5591
E03+T04-8	130	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E03+T04-9	147	0.3125	0.4916	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0968	0.2014
E03+T04-10	178	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E03+T04-11	192	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E03+T04-12	201	**0.5000**	**0.6931**	0.0494	0.1181	**0.4984**	**0.6915**	0.3412	0.5247	0.4316	0.6231
E03+T04-13	223	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.1591	0.2958	0.4725	0.6653
E03+T04-14	234	0.2733	0.4452	0.0974	0.2024	0.2552	0.4230	0.0548	0.1283	0.1780	0.3223
E03+T04-15	245	0.4142	0.6047	0.0000	0.0000	0.4444	0.6365	0.3796	0.5674	0.4932	0.6863
E03+T04-16	255	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E03+T04-17	321	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E03+T05-01	079	0.2321	0.3939	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0726	0.1607
E03+T05-02	082	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1944	0.3446
E03+T05-03	089	0.4832	0.6763	**0.5000**	**0.6931**	0.0000	0.0000	0.0000	0.0000	0.4259	0.6171
E03+T05-04	116	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0285	0.0756
E03+T05-05	122	0.4416	0.6336	0.4142	0.6047	0.0000	0.0000	0.1889	0.3372	0.3170	0.4970
E03+T05-06	130	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1944	0.3446
E03+T05-07	146	0.2321	0.3939	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0726	0.1607
E03+T05-08	154	0.2321	0.3939	0.3492	0.5337	**0.4930**	**0.6862**	0.0655	0.1481	0.3292	0.5110
E03+T05-09	162	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1623	0.3004
E03+T05-10	176	0.4746	0.6675	0.0494	0.1181	0.4571	0.6496	0.0000	0.0000	**0.4973**	**0.6905**
E03+T05-11	188	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1623	0.3004
E03+T05-12	206	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1180	0.2351
E03+T05-13	223	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0285	0.0756
E03+T05-14	233	0.1439	0.2740	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0430	0.1057
E03+T05-15	244	0.4746	0.6675	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.2849	0.4592
E03+T05-16	264	**0.4954**	**0.6886**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2610	0.4301
E03+T05-17	276	0.4832	0.6763	0.0000	0.0000	0.3750	0.5623	0.0000	0.0000	0.4460	0.6381
E03+T05-18	287	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1944	0.3446
E03+T05-19	302	0.0000	0.0000	0.2733	0.4452	0.2321	0.3939	0.0000	0.0000	0.1408	0.2694

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E03+T05-20	318	0.4746	0.6675	0.3472	0.5314	0.3750	0.5623	**0.4880**	**0.6811**	0.4552	0.6477
E03+T05-21	335	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1180	0.2351
E03+T05-22	353	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2928	0.4686
E03+T05-23	374	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1180	0.2351
E03+T05-24	405	0.4746	0.6675	0.4325	0.6240	0.3750	0.5623	0.0000	0.0000	0.4877	0.6808
E03+T05-25	434	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1180	0.2351
E03+T05-26	476	0.4746	0.6675	0.0000	0.0000	0.3750	0.5623	0.0000	0.0000	0.2761	0.4486
E03+T06-01	084	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E03+T06-02	090	0.3832	0.5714	**0.4954**	**0.6886**	0.0000	0.0000	0.0000	0.0000	0.4484	0.6406
E03+T06-03	126	0.4649	0.6576	0.3472	0.5314	0.2083	0.3631	0.4444	0.6365	**0.4997**	**0.6928**
E03+T06-04	146	0.2321	0.3939	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0680	0.1526
E03+T06-05	167	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E03+T06-06	183	0.4142	0.6047	0.0494	0.1181	0.4832	0.6762	0.4832	0.6762	0.4630	0.6557
E03+T06-07	205	0.0494	0.1181	0.0000	0.0000	0.0000	0.0000	0.3796	0.5674	0.1250	0.2457
E03+T06-08	212	**0.4954**	**0.6886**	0.0000	0.0000	0.3603	0.5461	0.4832	0.6762	0.4173	0.6080
E03+T06-09	229	0.0974	0.2024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0266	0.0715
E03+T06-10	240	0.1439	0.2740	0.2733	0.4452	**0.4880**	**0.6811**	**0.4832**	**0.6762**	0.4230	0.6140
E03+T06-11	262	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2552	0.4230	0.0686	0.1536
E03+T06-12	354	0.4954	0.6886	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2467	0.4124
E03+T06-13	381	0.4944	0.6876	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.2907	0.4661
E03+T06-14	434	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E04+T02-01	102	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2101	0.3654
E04+T02-02	136	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2101	0.3654
E04+T02-03	174	0.4325	0.6240	0.0000	0.0000	**0.4507**	**0.6430**	0.0000	0.0000	0.2716	0.4431
E04+T02-04	180	0.3832	0.5714	0.0000	0.0000	0.3964	0.5856	0.0000	0.0000	0.2272	0.3876
E04+T02-05	190	0.3832	0.5714	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1283	0.2508
E04+T02-06	213	0.1439	0.2740	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0408	0.1012
E04+T02-07	217	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1121	0.2259
E04+T02-08	225	0.4746	0.6675	0.0000	0.0000	0.4507	0.6430	0.0000	0.0000	0.2965	0.4729
E04+T02-09	232	0.3472	0.5314	0.0000	0.0000	0.3575	0.5429	**0.3796**	**0.5674**	0.4846	0.6777
E04+T02-10	260	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1852	0.3322
E04+T02-11	278	0.3492	0.5337	**0.0494**	**0.1181**	0.0000	0.0000	0.0000	0.0000	**0.4978**	**0.6909**
E04+T02-12	290	**0.4954**	**0.6886**	0.0000	0.0000	0.0000	0.0000	0.3412	0.5247	0.4927	0.6858
E04+T02-13	326	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1544	0.2892
E04+T02-14	339	0.4954	0.6886	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2495	0.4158
E04+T02-15	345	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1544	0.2892
E04+T02-16	393	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1121	0.2259
E04+T02-17	434	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2101	0.3654
E04+T02-18	448	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1852	0.3322
E04+T03-01	070	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E04+T03-02	076	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1831	0.3293
E04+T03-03	084	0.4416	0.6336	0.0000	0.0000	0.2552	0.4230	**0.4444**	**0.6365**	0.3214	0.5020
E04+T03-04	126	**0.4954**	**0.6886**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2467	0.4124
E04+T03-05	144	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E04+T03-06	159	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E04+T03-07	169	0.3125	0.4916	0.0000	0.0000	0.4444	0.6365	0.0000	0.0000	**0.4127**	**0.6031**
E04+T03-08	179	0.4325	0.6240	0.0000	0.0000	0.3603	0.5461	0.0000	0.0000	0.2394	0.4032
E04+T03-09	192	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.3603	0.5461	0.2657	0.4359
E04+T03-10	225	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E04+T03-11	238	0.4325	0.6240	0.0000	0.0000	**0.4984**	**0.6915**	0.0000	0.0000	0.3138	0.4932
E04+T03-12	251	0.4142	0.6047	0.0000	0.0000	0.4444	0.6365	0.0000	0.0000	0.3896	0.5783
E04+T04-01	091	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1526	0.2866
E04+T04-02	105	0.4325	0.6240	0.4746	0.6675	0.4444	0.6365	0.0000	0.0000	0.3887	0.5773
E04+T04-03	111	**0.4954**	**0.6886**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2467	0.4124
E04+T04-04	126	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E04+T04-05	131	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2775	0.4502
E04+T04-06	142	0.4325	0.6240	0.1889	0.3372	0.1591	0.2958	0.1591	0.2958	0.3740	0.5612
E04+T04-07	146	0.0000	0.0000	**0.4944**	**0.6876**	**0.4984**	**0.6915**	0.4832	0.6762	0.4841	0.6772

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E04+T04-08	152	0.4746	0.6675	0.1889	0.3372	0.2083	0.3631	0.1591	0.2958	0.3623	0.5483
E04+T04-09	157	0.4649	0.6576	0.0000	0.0000	0.4984	0.6915	0.0000	0.0000	0.4015	0.5911
E04+T04-10	176	0.3472	0.5314	0.0000	0.0000	0.4444	0.6365	0.0000	0.0000	0.2376	0.4009
E04+T04-11	185	0.0974	0.2024	0.4746	0.6675	0.4832	0.6762	0.0000	0.0000	**0.4946**	**0.6878**
E04+T04-12	206	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.3796	0.5674	0.4153	0.6059
E04+T04-13	216	0.2733	0.4452	0.4416	0.6336	0.2083	0.3631	**0.4984**	**0.6915**	0.4056	0.5956
E04+T04-14	225	0.4325	0.6240	0.4832	0.6763	0.3603	0.5461	0.3603	0.5461	0.4553	0.6478
E04+T04-15	242	0.4944	0.6876	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2077	0.3623
E04+T04-16	257	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E04+T04-17	265	0.4416	0.6336	0.0000	0.0000	0.3796	0.5674	0.0000	0.0000	0.2507	0.4174
E04+T04-18	283	0.4746	0.6675	0.0000	0.0000	0.4832	0.6762	0.0000	0.0000	0.3183	0.4985
E04+T04-19	302	0.4325	0.6240	0.4944	0.6876	0.4832	0.6762	0.4694	0.6623	0.4940	0.6871
E04+T04-20	312	0.4944	0.6876	0.0000	0.0000	0.4832	0.6762	0.0000	0.0000	0.3368	0.5197
E04+T04-21	323	0.4832	0.6763	0.0000	0.0000	0.3796	0.5674	0.4880	0.6811	0.3922	0.5811
E04+T04-22	363	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E04+T04-23	380	0.3472	0.5314	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1108	0.2238
E04+T04-24	440	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E04+T05-01	076	0.3536	0.5386	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1095	0.2218
E04+T05-02	092	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1811	0.3265
E04+T05-03	098	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1509	0.2841
E04+T05-04	104	0.1023	0.2104	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0270	0.0724
E04+T05-05	115	**0.4966**	**0.6898**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2055	0.3594
E04+T05-06	126	0.4923	0.6855	0.0000	0.0000	**0.4444**	**0.6365**	0.0000	0.0000	**0.3458**	**0.5299**
E04+T05-07	142	0.4789	0.6719	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1811	0.3265
E04+T05-08	164	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E04+T05-09	193	0.3536	0.5386	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1095	0.2218
E04+T05-10	225	0.4384	0.6302	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1509	0.2841
E04+T06-01	101	0.4746	0.6675	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1852	0.3322
E04+T06-02	130	**0.5000**	**0.6931**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2311	0.3927
E04+T06-03	148	0.0494	0.1181	0.0494	0.1181	0.0000	0.0000	0.0000	0.0000	0.0266	0.0715
E04+T06-04	165	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1544	0.2892
E04+T06-05	192	0.4325	0.6240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1544	0.2892
E04+T06-06	222	0.4649	0.6576	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2804	0.4537
E04+T06-07	262	0.4746	0.6675	**0.3472**	**0.5314**	**0.2552**	**0.4230**	**0.2686**	**0.4394**	**0.4934**	**0.6866**

Table 4.34. The scorable size range, Number of AFLP bands (N), Nei's (1973) gene diversity (h) and Shannon's Information index (I) values in different populations and across the populations with various primers combinations

Primer Comb.	Size Range (bps)	N	Red Jungle Fowl		White Leghorn		Aseel		Red Cornish		Overall	
			h	I	h	I	h	I	h	I	h	I
E01+T02	057-440	22	0.3396	0.5051	0.0284	0.0447	0.0628	0.0937	0.0619	0.0880	0.2091	0.3478
E01+T03	116-440	16	**0.4552**	**0.6437**	0.0622	0.0864	0.1169	0.1651	0.0000	0.0000	0.2746	0.4413
E01+T06	075-420	14	0.3763	0.5468	0.0885	0.1359	0.0712	0.1066	0.1044	0.1550	0.2554	0.4001
E02+T01	065-288	15	0.3536	0.5160	0.0519	0.0764	0.1068	0.1571	0.0764	0.1112	0.2193	0.3627
E02+T02	056-342	13	0.3679	0.5255	0.0000	0.0000	0.0930	0.1364	0.0000	0.0000	0.1708	0.2933
E02+T03	074-253	16	0.3663	0.5236	0.0305	0.0426	0.1196	0.1788	0.1194	0.1676	0.2424	0.3927
E02+T04	084-345	17	0.3814	0.5523	0.0512	0.0775	0.1381	0.2038	0.0523	0.0749	0.2344	0.3790
E02+T05	100-415	17	0.4116	0.5990	0.0793	0.1132	0.1519	0.2275	0.0721	0.1116	0.2771	0.4411
E02+T06	116-330	11	**0.3154**	**0.4711**	0.0000	0.0000	0.1744	0.2564	0.1201	0.1788	0.2341	0.3772
E03+T01	092-470	23	0.4068	0.5900	**0.1826**	**0.2718**	0.2197	0.3156	0.0410	0.0577	0.2856	0.4475
E03+T02	170-384	14	0.3887	0.5588	0.0689	0.0965	0.0888	0.1293	0.0537	0.0838	0.2305	0.3771
E03+T03	087-360	12	0.4204	0.6066	0.0833	0.1155	0.1316	0.1942	0.0300	0.0455	0.2281	0.3785
E03+T04	055-321	17	0.3499	0.5200	0.0495	0.0814	0.1844	0.2719	0.0837	0.1293	0.2701	0.4162
E03+T05	079-476	**26**	0.3636	0.5348	0.1040	0.1569	0.1030	0.1530	0.0290	0.0448	0.2250	0.3660
E03+T06	084-434	14	0.3381	0.4949	0.1080	0.1653	0.1100	0.1619	**0.1806**	**0.2611**	0.2625	0.4119
E04+T02	102-448	18	0.4165	0.6033	0.0027	0.0066	0.0920	0.1341	0.0400	0.0607	0.2346	0.3843
E04+T03	070-251	12	0.4249	0.6147	0.0000	0.0000	0.1669	0.2445	0.0671	0.0986	0.2462	0.4045
E04+T04	091-440	24	0.3772	0.5473	0.1350	0.1956	**0.2297**	**0.3360**	0.1249	0.1840	**0.2974**	**0.4585**
E04+T05	076-225	10	0.3633	0.5267	0.0000	0.0000	0.0444	0.0637	0.0000	0.0000	**0.1461**	**0.2627**
E04+T06	101-262	7	0.4041	0.5788	0.0567	0.0928	**0.0365**	**0.0604**	0.0384	0.0628	0.2179	0.3593

Table 4.35. Population-specific AFLP markers in Red jungle Fowl (RJF), White Leghorn (WLH), Aseel (AS) and Red Cornish (RC) with various AFLP primer combinations

Primer Combination	Population-specific AFLP fragments (fragment number with their respective sizes (in bps) and allele frequencies per population			
	RJF	WLH	AS	RC
E01+T02	01(057) (0.0253) 02 (061) (0.0780) 09 (149) (0.2254) 10 (162) (0.1056) 17 (260) (0.0513)			
E01+T03	09(265) (0.3292)			
E02+T02	03 (108) (0.0780)			
E02+T03			08 (142) (0.4226)	
E02+T04	08 (204) (0.1340)			
E02+T06	04 (152) (0.1340) 08 (277) (0.0513)			
E03+T02	10 (311) (0.0513)			05 (230) (0.1181)
E03+T03	03 (126) (0.2745) 09 (218) (0.0823)			
E03+T04	03 (082) (0.0780) 09 (147) (0.1398)			
E03+T05	01 (079) (0.1340) 04 (116) (0.0513) 07 (146) (0.1340) 13 (223) (0.0513) 14 (233) (0.0780)			
E03+T06	04 (146) (0.1340) 09 (229) (0.0513)			11 (262) (0.1502)
E04+T02	05 (190) (0.2584) 06 (213) (0.0780)			
E04+T05	04 (104) (0.0541)			
Total	**26**	**0**	**1**	**2**

Table 4.36. Coefficient of genetic differentiation (G_{ST}) (above diagonal) and Gene flow (Nm) (below diagonal) estimated among populations using AFLP markers

	RJF	WLH	AS	RC
RJF	-	0.2502	0.2032	0.2723
WLH	1.4984	-	0.2342	0.3104
AS	1.9600	1.6348	-	0.2077
RC	1.3362	1.1106	1.9069	-

Table 4.37. Genetic diversity statistics of all populations with AFLP markers

Parameters	RJF	WLH	AS	RC	Overall
Sample size	20	20	18	18	76
Total bands					318
Polymorphic bands	302	60	105	57	309
Polymorphism (%)	95	19	33	18	97
Population- specific bands	26	-	1	2	29
G_{ST} (across all 318 loci)					0.3246
Nm (across all 318 loci)					1.0405
h (across all 318 loci)	0.3805	0.0669	0.1287	0.0656	0.2427
I (across all 318 loci)	0.5529	0.0993	0.1889	0.0968	0.3910

Table 4.38. Genetic identity estimated as Nei (1972) (above diagonal) and genetic distance (below diagonal) among populations using AFLP markers

	RJF	WLH	AS	RC
RJF	-	0.8246	0.8377	0.8017
WLH	0.1928	-	0.9343	0.9361
AS	0.1771	0.0680	-	0.9442
RC	0.2210	0.0660	0.0575	-

Discussion

Discussion

In the present study, we attempted to understand the genetic polymorphism between red jungle fowl and the domestic chicken breeds, represented by three breeds i.e. White Leghorn (WLH) as egg-type, Aseel (AS) as game-type and Red Cornish (RC) as meat-type breeds by using two type of DNA markers i.e. microsatellite and AFLP markers and to identify population specific private alleles for Indian red jungle fowl (*Gallus g. murgi*) in order to develop molecular standards for the purity of Red Jungle Fowl. Various parameters of genetic diversity measurements were studied and phylogenetic relationship of different domestic chicken breed with RJF was also studied. We addressed 76 Indian birds for analysis that includes RJF (*G. g. murgi*) as wild fowl and WLH, AS and RC to represent domestic chicken breeds. In this study, we used two type of DNA markers, microsatellite as co-dominant type and AFLP as dominant type. Microsatellite are locus specific markers whereas AFLP markers are multilocus. But both type of marker systems are highly reproducible and very informative.

5.1. Microsatellite Analysis

Microsatellites are widely dispersed throughout eukaryotic genomes and are often highly polymorphic due to variation in the number of the repeat motif (core sequences) generated by unequal crossover between the repeat units during meiosis among populations/ individuals. The most common way to detect microsatellites is to design PCR primers that are unique to one locus in the genome and that base pair on either side (flanking regions) of the repeated portion. Microsatellites have been proposed as the best markers for genetic diversity studies in chickens because of their abundant, even distribution in the genome, co-dominantly inherited, high polymorphism and ease of genotyping.

5.1.1. Genetic diversity analysis

Genetic diversity can be evaluated by the number of alleles per locus, Allelic size range, expected heterozygosity, observed heterozygosity and PIC. In present study, all the 25 microsatellite loci were polymorphic within as well between the populations, except Locus ADL120 in WLH and RC, where only one allele i.e. 156

bp in WLH and 152 bp in RC was observed. Hillel *et al.,* (2003) also reported that at all the microsatellite loci, all the populations are not polymorphic. The frequency of polymorphic population ranged from 0.69 to 1.00. In our study, the frequency of polymorphic population ranged from 0.96 to 1.00. In total, 475 alleles were found at the 25 loci across the RJF and 3 chicken breeds. Across the population, the number of alleles at each locus ranged from 5 (MCW111) to 43 (LEI212) with an average 19 alleles per locus. Population-wise the mean allele number ranged from 7.08 (WLH and RC) to 9.92 (RJF). Barker (1994) suggested that microsatellite loci used in studies of genetic distance should have no fewer than four alleles in order to reduce the standard errors of distance estimates; thus, the microsatellites used in this study were suitable for genetic diversity analysis. More alleles were found at each locus in this study as compared to the earlier reports. Number of alleles as well as average number of loci reported by different workers varied widely. Kaiser *et al.,* (2000) reported the average number of alleles per primer to be 2.8 and 2.9 in two chicken populations. While Romanov and Weigend (2001) compared 20 chicken populations of different origin by typing 14 microsatellites and reported 2 and 21 alleles with the mean of 11.2 alleles per locus. However Hillel *et al.,* (2003) reported 4 (MCW98) to 23 (LEI192) alleles with an average of 9.6 alleles per loci, across the populations. Cuc *et al.,* (2006) reported 2 to 23 alleles with an average of 6.41 alleles per locus. Similarly, Qu *et al.,* (2006) reported 6 to 51 alleles in 78 indigenous chicken breeds at 27 microsatellite loci. Haunshi and Sharma (2006) reported 2 to 7 alleles with an average of 3.3 alleles per marker. Tomar *et al.,* (2007) also reported 3 – 6 alleles in RC, AS, RJF and WLH at 5 microsatellite loci. Average number of alleles was 18.74 per locus that is more similar to the average number of alleles presented in our study. Bao *et al.*, (2008) reported 2 (MCW 103) to 30 (LEI 234) alleles across the populations, with an average of 9.86 ± 6.36 alleles. This vast difference in number of alleles at different locus might be due to difference in microsatellite markers and the population by various workers.

Heterozygosity is an appropriate measure of genetic variability in a population. High heterozygosity was also reported in the present study in Red Jungle fowl as well as different chicken breeds. Takezaki and Nei (1996) determined that, for markers to be useful for measuring genetic variation, they should have a mean heterozygosity of between 0.3 and 0.8 in the population. In our study, expected heterozygosity (H_E) per locus ranged from 0.7285 (MCW111) and 0.9450 (LEI212),

whereas across all loci, it was 0.8830. Population-wise mean heterozygosity ranged from 0.6946 in WLH to 0.7975 in RJF. High heterozygosity revealed high polymorphism in the population at these microsatellite loci. Therefore, the markers used in this study were appropriate for measuring genetic variation.

Similarly, The PIC value per locus ranged from 0.6814 (MCW111) and 0.9426 (LEI212), whereas across all loci, it was 0.8715. Population-wise mean PIC value ranged from 0.6576 in WLH to 0.7716 in RJF. According to Botstein *et al.,* (1980) genetic markers showing PIC values higher than 0.5 are normally considered as informative in population genetic analysis. So, the markers used in this study were highly informative.

The estimates of expected heterozygosity and PIC varied widely in the literature. Cheng *et al.*, (2003) reported that average heterozygosity in the Shouguang chicken was the lowest (0.3327), and that in other breeds was also less than 0.40. The PIC values ranged from 0.617 (Shouguang chicken) to 0.703 (Laiwu Black chicken). Pandey *et al.,* (2003) reported mean heterozygosity over all loci was 0.62, 0.62, and 0.61 for Nicobari, Miri, Aseel poultry, respectively. Li *et al.,* (2004) reported the heterozygosity of microsatellite markers in the range of 0.108 to 0.765 in egg type chicken. Tu *et al.,* (2005) used 30 microsatellite markers to detect the genetic diversity of 8 indigenous chicken breeds in Sichuan and found that the mean heterozygosity of 8 chicken breeds was all over 0.5. The highest was the Luning chicken (0.681), and the lowest was the Jiuyuan Dark chicken. Qu *et al.,* (2006) reported that the heterozygosity (H) values of the 78 chicken breeds were all more than 0.5. The average H value (0.622) and PIC (0.573) of these breeds suggested that the Chinese indigenous chickens possessed more genetic diversity than that reported in many other countries. Tadano *et al.,* (2007) evaluated the genetic diversity and relationships of 9 native Japanese long-tailed chicken breeds. While the mean expected heterozygosity ranged from 0.293 (Koeyoshi) to 0.545 (Satsumadori), mean PIC ranged 0.250 (Koeyoshi) to 0.478 (Satsumadori), respectively. Bao *et al.,* (2008) reported H_E in the range of 0.2915 (MCW98) to 0.9205 (LEI234) and over all loci was 0.6663. Within population, they reported the lowest value of H_E (0.4532) with Gushi chicken breed and the highest (0.6442) for Wannan Three-yellow population. While at locus MCW111, they reported H_E (0.7287) was more similar to H_E (0.7285) presented in our study. In a study on Indian birds, Kanginakudru *et al.,* (2008) evaluated maen heterozygosity ranged from 0.481 (Kaleser population of RJF) to

0.600 (Birshi Kargah population of RJF). Kaya and Yildiz (2008) reported population-wise H_E was higher in the Dinizli breed (0.656) than in the Gerze breed (0.475) with mean H_E of 0.665 and PIC values were 0.599 and 0.426 for Dinizli and Gerze breeds, respectively with mean of 0.610 across all loci. In a recent study on Qingyuan chicken, Fang *et al.,* (2009) reported the H_E in the range between 0.500 and 0.839, with mean of 0.685 across all loci and the PIC value in the range between 0.375 and 0.818, with mean of 0.618 across all loci.

The range of observed heterozygosity (H_O) in this study was between 0.1486 (ADL120) and 0.6842 (LEI234), whereas across the all loci, it was 0.4344. Population-wise mean observed heterozygosity ranged from 0.3822 in WLH to 0.4565 in RJF. Although varying among populations, mean observed heterozygosity was lower than the mean expected heterozygosity for all the populations. Kaya and Yildiz (2008) evaluated the mean H_O was 0.380 in the Gerze breed and 0.508 in the Denizli breed.

These results clearly revealed that all the markers used in present study were moderate to highly polymorphic, hence were capable of detecting the underlying genetic variability within as well as between the populations.

5.1.2. Population specific alleles

A total of 242 population specific alleles with allelic frequency ranged from 0.0250 to 0.8333 were detected across the RJF and three domestic chicken breeds with all the 25 microsatellite loci. Out of these 242 population specific alleles, 103 were specific to RJF, 44 were specific to WLH, 56 were specific to AS and 39 were specific to RC. Out of 242 population specific alleles, majority of the alleles (~75 %) were present in low to very low frequency and only 61 alleles were found in a frequency of equal to or more than 0.10. Among these 61 alleles, we found 29 population specific alleles in the range of allelic frequency 0.100 to 0.500 in RJF, 12 in RC (0.111 to 0.647), 10 in WLH (0.100 to 0.575) and also 10 in AS (0.100 to 0.833). Among the 29 RJF specific alleles, only 4 alleles, i.e. ADL120-178 bp, ADL 265-114 bp, MCW111-104 bp and MCW111-106 bp seemed to have some practical importance as these alleles are present in very good allelic frequency range of 0.500 to 0.526. In domestic chicken breeds, three alleles i.e. LEI243-186 bp in WLH, LEI214-140 bp in AS and LEI229-193 bp in RC were more important from the breed

identification point of view. Considering all the chicken population together, there are 226 alleles, which are present in domesticated chicken, but absent in RJF.

Hillel *et al.*, (2003) using the 22 microsatellite markers in the 52 chicken populations, 32 private alleles were identified. RJF had eight private alleles that were absent in the domesticated gene Pool, while taken together, the 50 domesticated populations, 91 alleles were missing in the two RJF populations. Zhou and Lamont (1999) evaluated line-specific alleles among breeds and lines i.e. Leghorn, Jungle Fowl, Fayoumi and Spanish breeds. They reported 7 breed specific alleles in Fayoumi breed, 19 line specific alleles in line UCD-001 and 24 line specific alleles in other different lines with 41 microsatellite loci. Wardecka *et al.,* (2004) determined microsatellite polymorphism in Rhode Island Red (RIR) and Sussex (SX) chickens, divergently selected over six generations for high (H) or low (L) incidence of skeletal defects in embryos (30.7% for H lines, 3.7% for L lines). The polymorphism analysis covered 15 microsatellite markers within four lines (a total of 60 individuals). Eight alleles were identified as specific to H lines and six alleles as specific to L lines. Nakamura *et al.*, (2006) used 25 microsatellite markers to identify the polymorphism between 4 strains of Nagoya breed (native to Japan) from other breeds and commercial stocks of chicken. In these strains, 5 of the markers (ABR0015, ABR0257, ABR0417, ABR0495, and ADL0262) had a single allele, while no other chicken breeds and hybrids had the same allele combination as the Nagoya breed strains. Hence, these 5 microsatellite markers provide a practical method to accurately discriminate the Nagoya breed from other chicken breeds. Rikimaru and Takahashi (2007) successfully discriminated the Hinai-jidori chicken from other chickens on the basis of Hinai-jidori specific alleles at 14 marker loci i.e. ABR1003, ADL0250, ABR0241, ABR0311, ABR1004, ABR1013, ABR0633, ABR1005, ABR0089, ABR1007, ABR1001, ABR1009, ABR1010, and ABR1011. Hence the microsatellite assay can effectively be used in discriminating a breed / line from other populations by identified population specific alleles.

5.1.3. Genetic Differentiation and Gene flow

Genetic differentiation was examined by fixation indices F_{IS}, F_{IT} and F_{ST} for each locus across populations and among population pairs. The statistics F_{IS} is an estimate of variation within population that measures the reduction in heterozygosity in an individual due to nonrandom mating within its subpopulation. The F_{IT} is the

overall inbreeding coefficient of an individual relative to the total population. This includes the contribution due to nonrandom mating within subpopulations (F_{IS}) and due to population subdivision (F_{ST}). F_{ST} is an estimate of variation due to differences among populations, which is the reduction in heterozygosity of a subpopulation due to genetic drift.

In present study, the F_{IS} estimates were positive and high (0.1449 to 0.6615) with an average of 0.4047. These positive estimates suggest the deficit of heterozygotes at each locus and also across all the loci. Since the statistics F_{IS} is an estimate of variation within population that measures the reduction in heterozygosity in an individual due to nonrandom mating within its subpopulation, the positive and high estimates of F_{IS} might be expected in the populations being maintained as a pedigreed closed flock and are under some kind of selection. Kaya and Yildiz (2008) reported the mean F_{IS} of 0.301 across all the loci among native Turkish chicken, while other workers have reported comparatively much lower F_{IS} estimates. Dai *et al.,* (2006) reported F_{IS} ranging from –0.1172 to 0.1815 in five chicken populations i.e. New Yangzhou, Rugao, Jiangchun, Wan-Nan and the Cshiqishi using 5 Micro satellite markers. Yu *et al.,* (2006) reported the F_{IS} ranging from 0.216 to –0.831 with an average of -0.357 in 12 Chinese indigenous chicken breeds using 30 microsatellite markers. Cuc *et al.,* (2006) reported that the means of the F_{IS} of 0.044 (-0.007 to 0.196) in H'mong chickens, a local breed in the mountainous areas of Northern Vietnam. The low positive to moderately negative values of F_{IS} in these populations due to the reason that these populations were the native populations, sampled from their natural in habitat and were not the closed flock populations.

In present study, the F_{IT} estimates ranged from 0.2649 to 0.8024 at various loci, while across all the loci, the estimate was 0.5068. These estimates were high in comparison to those reported for different indigenous chicken. Dai *et al.,* (2006) reported the F_{IT} values in 5 indigenous chicken varying from –0.0908 to 0.2111. In H'mong chickens (local breed in the mountainous areas of Northern Vietnam), Cuc *et al.,* (2006) reported mean F_{IT} of 0.069. *Bao et al.,* (2008) reported the F_{IT} ranging from 0.079 to 0.308 at different loci, with an average of 0.179 across all the loci within the population of 2 sub-species of RJF and 14 Chinese domestic chicken breeds.

The F_{ST} estimates, which also represent the fixation coefficient of sub populations ranged from 0.0580 (ADL254) to 0.5530 (ADL120) with a mean of

0.1716 across loci. This mutilocus F_{ST} value (0.1716) indicated that around 17% of total genetic variation was explained by differences among populations while the remaining 83% corresponding to differences among individuals within population. *Bao et al.,* (2008) evaluated the fixation coefficient (F_{ST}) of subpopulations within the population of 2 sub-species of RJF and 14 Chinese domestic chicken breeds and reported mean F_{ST} of 0.167 across loci. This F_{ST} value (0.167) was much closer to the F_{ST} value observed in present study. However, several other workers reported much lower F_{ST}. Dai *et al.,* (2006) reported F_{ST} ranging from 0.008 to 0.0415 in five chicken populations i.e. New Yangzhou, Rugao, Jiangchun, Wan-Nan and the Cshiqishi using 5 Micro satellite markers. Similarly, Cuc *et al.,* (2006) reported that the means of the F_{ST} of 0.026 (-0.036 to 0.196) in H'mong chickens, a local breed in the mountainous areas of Northern Vietnam.

Pair-wise F_{ST} among all 4 populations ranged from 0.1027 to 0.1443. The F_{ST} estimates between RJF and domestic chicken breeds were lower (0.1027 to 0.1224) in comparison to those among the domestic chicken breeds (0.1278 to 0.1443). Among the domestic chicken breeds, RJF showed lowest F_{ST} with AS (0.1027) and highest with RC i.e. 0.1224. Kanginakudru *et al.,* (2008) reported the F_{ST} value ranging from 0.246 to 0.311 between *G. g. murgi* and *G. g. domesticus* , while it ranged from 0.141 to 0.164 between domestic chicken groups. The estimates between *G. g. murgi* and *G. g. domesticus* were higher than those estimated in present study, while the estimates between domestic chicken were comparable to our estimates.

Among Red jungle fowl and domestic chicken breeds, in present study, the gene flow (Nm) varied from 0.2021 (ADL120) to 4.0612 (ADL254) with a mean of 1.2070 across all loci. Pair-wise gene flow between RJF and domestic chicken populations, the gene flow estimates ranged from 1.4822 to 2.1840. The gene flow estimates between RJF and domestic chicken breeds were higher 1.7921 to 2.1840) in comparison to those among the domestic chicken breeds (1.4822 – 1.7057). Among the domestic chicken breeds, RJF showed maximum gene flow estimates of 2.1840 with AS and lowest with RC i.e. 1.7921. Within the population of 2 sub-species of RJF and 14 Chinese domestic chicken breeds, *Bao et al.,* (2008) reported the gene flow value ranging from 0.583 to 4.750. Between the RJF (*Gallus gallus gallus*) and other native chicken breeds, the gene flow estimates ranged from 0.533 to 1.229, while this range was from 0.695 to 2.078 between other subspecies of RJF i.e. *Gallus gallus spadicieus* and native Chinese chicken breeds and both the estimates were

close to those reported in present study. Dai *et al.*, (2006) reported much higher gene flow i.e. 5.7741 to 30.2378 between the five chicken populations i.e. New Yangzhou, Rugao, Jiangchun, Wan-Nan and the Cshiqishi.

5.1.4. Genetic relatedness and phylogenetic analysis

Genetic distances between the populations were estimated using shared allele frequencies. Genetic distance between RJF and domestic chicken populations namely as WLH, AS and RC was observed 0.7562, 0.7689 and 0.8093 respectively. Within domestic chicken populations genetic distances was observed 0.7637 (between WLH and RC) followed by 0.7495 (between WLH and AS) and 0.7302 (between AS and RC). Wild RJF showed maximum genetic distance with RC and minimum genetic distance with WLH. Among domestic populations, maximum genetic distance was observed between WLH and RC and minimum between AS and RC.

The UPGMA dendrogram based on shared allele distances clearly clustered all 4 populations to understand relationships between RJF and domestic chicken breeds. Broadly, All four populations were divided in to two main clusters. The Wild RJF and WLH were placed in cluster-I and AS and RC were placed in cluster-II. As we see the WLH chicken grouped with RJF, it showed that the WLH (egg type) was much close to the wild RJF than the AS breed (game type) and RC breed (meat type) and on the other hand the RC breed (meat type) had close relationship with AS breed (game type). This was also proved earlier as described by Moiseyeva *et al.*, (2003) in their study. The pair-wise distances between wild RJF and other thee domestic breeds namely as WLH, AS and RC breeds were higher then the distances between domestic chicken breed pairs as between WLH and AS, between WLH and RC and between AS and RC.

Zhou and Lamont (1999) reported in their analysis that the polymorphism of 42 microsatellite loci was examined in 23 highly inbred fowl lines derived from the RJF, Leghorn, Fayoumi and Castellana Negra breeds. Genetic distances based on the proportion of shared alleles between jungle fowls and other lines were larger (1.12 to 5.38) as compared with distances between the domestic fowl lines (0.66 to 1.13).

Moiseyeva *et al.*, (2003) tried to understand the most important unsolved question that which type of domestic chicken is more close to RJF and, therefore, what types of domesticated fowls are the most ancient on the basis of several morphological characters and biochemical markers. They found greatest similarity

between *G. gallus* and the egg-type breeds of Mediterranean roots and/or true Bantams. Variable chicken breeds such as egg type, game type and meat type did not arise simultaneously. Probably, the first forms were of the Mediterranean egg-layer and/or Bantam types. The game type of chicken breeds might also derive directly from the wild progenitor or, rather, from the egg-type birds. The latest evolutionary lineage was the meat type that is suggested to have descended from game breeds.

Romanov and Weigend (2001) compared three RJF populations with 17 chicken populations of the Ukrainian, Russian, German and Australian origins by polymorphism of 14 microsatellite loci covering 11 linkage groups. As a result, the RJF formed a separate branch on the genetic relationship tree.

Kanginakudru *et al.,* (2008) in their analysis on 76 Indian birds reported a higher average Nei's genetic distance between *G. sonnerati* and *G. g. domesticus* (2.099) than the *G. g. murghi – G. g. domesticus* (1.695).

5.2. AFLP Analysis

Amplified fragment length polymorphism (AFLP) has been widely used to study whole-genome polymorphisms in both eucaryotes and prokaryotes. It involves PCR amplification and selective detection of fragments between neighboring, frequently distributed restriction sites in the organism used for the study (Savelkoul *et al.,* 1999). AFLP markers offer several advantages over the other currently used DNA markers such as Microsatellites and SNPs. Foremost among these is that the AFLP technology can be easily adapted to the DNA of any organism without the need for prior sequence knowledge and, hence, has a relatively low start-up cost (Vulsteke *et al.,* 2007). Two limited sets of AFLP primers are sufficient to generate a large number of different primer combinations (PCs), each of which will yield unique fingerprints.

In this study we used two restriction enzymes a rare cutter (*Eco*RI) and a frequent cutter (*Taq*I). The frequent cutter was used to generate short DNA fragment that are in the 50- 500 bp length range resolvable by electrophoresis. The rare cutter is used to limit the number of fragments that can be amplified and, hence, to define the number of effective AFLP amplicons. Selection of restriction enzymes is based on the efficiency of polymorphism detection, the genome coverage and AFLP marker distribution. Genome coverage and AFLP marker distribution are mainly determined by the AT-/CG- content of the DNA. For CG-rich genomes like in poultry, CG-rich restriction enzymes such as *Taq*I (recognition site TCGA) is more appropriate

restriction enzymes (Vuylsteke *et al.,* 2007). The *Eco*RI/*Taq*I have been most commonly used in poultry (Herbergs *et al.,* 1999; Knorr *et al.,* 1999).

5.2.1. Genetic diversity analysis

A total of 318 scorable AFLP bands in the range of 50-500 bp were detected across the populations with 20 *Eco*RI/*Taq*I primer combinations. Out of which, 309 (97.17%) were polymorphic bands. The mean number of polymorphic bands across all 4 populations was 15.4 per primer combination (PC). The extant of polymorphism varied within the populations as well as with each primer combinations. In RJF population, a total of 311 bands could be scored. Out of which, 302 (97%) were found polymorphic. Among the primer combinations the extant of polymorphism was 85% to 100%. However, challenges in AFLP analysis arose from their dominant scoring and the low level of polymorphism of some primer combinations among domestic chicken breeds. In WLH population, a total of 270 bands could be scored. Out of which, 60 (22%) were found polymorphic. In AS population, a total of 282 bands could be scored. Out of which, 105 (37%) were found polymorphic. In RC population, a total of 281 bands could be scored. Out of which, 57 (20%) were found polymorphic. The mean polymorphic bands across all 20 PCs were 15.1 in RJF, 3.0 in WLH, 5.2 in AS and 2.8 in RC. The number of polymorphic bands per primer combination and across all combinations was found very high in RJF than the domestic chicken breeds.

Herbergs *et al.,* (1999) reported a total of 475 polymorphic AFLP markers using 57 *Eco*RI/*Taq*I primer pair combinations in a population consisting of four families with a total of 183 F_2 individuals. The number of AFLP polymorphisms detected per primer pair varied from 0 to 21, with an average of 8.5 AFLP markers per primer pair. De Marchi *et al.*, (2005) used AFLP to detect genetic variation in four indigenous chicken breeds from the Veneto region of Italy. The three primer combinations revealed 188 bands; 70 of them were distinct AFLP polymorphisms (37%), with an average of 23.3 ± 1.7 markers per primer pair and a range from 21 to 25. The number of polymorphisms observed within breeds varied from 34 (broiler, with an average number per primer pair of 11.3 ± 0.5) to 43 (Padovana, with an average of 14.3 ± 2.6).

Mekchay *et al.,* (2005) used 15 *Eco*RI/*Taq*I primer combinations were used to generate AFLP markers among 10 pooled DNA samples belonging to either slow

(Thai native chicken) or fast-growing strains (broiler). A total 493 of AFLP bands were detected of which 199 revealed as polymorphic bands. An average AFLP polymorphic bands were 13.27 markers and a range between 3-29 marker per primer pair. Gao *et al.,* (2007) used six AFLP primer combinations to detect genetic variation in 12 Chinese indigenous chicken breeds. The six primer combinations, giving, on average, 46.5 polymorphic markers detected per primer combination, generated a total of 279 polymorphic bands.

The average number of polymorphic bands per primer combination presented in our study (15.4) was more similar to reported by Mekchay *et al.,* (2005). However, higher than reported by Herbergs *et al.,* (1999) and lower then reported by Gao *et al.,* (2007). In our study we had a high polymorphism in RJF that refers to its wild nature. The extant of polymorphism presented in three domestic chicken breeds in our study was more similar to reported by De Marchi *et al.*, (2005) in four different indigenous chicken breeds from the Veneto region of Italy.

Nei's genetic diversity was measured as per Nei (1973) and Shannon's Information Index was measure as per Shannon (1949) at each locus as well as across all the loci in each population i.e. RJF, WLH, AS and RC. Mean Nei's genetic diversity (h) across all loci generated by all 20 PCs was maximum in RJF (0.3850) followed by in AS (0.1287). In WLH and RC, the h estimates were quite low (0.0669 and 0.0656, respectively). Similarly the means of Shannon's Information Index (I) across all loci generated by all 20 PCs was maximum in RJF (0.5529), followed by AS (0.1889) and were quite low in WLH and RC (0.0993 and 0.0968, respectively). Mean Nei's genetic diversity (h) and mean Shannon's Information Index (I) across all 318 loci generated by all 20 PCs and across all populations were 0.2427 and 0.3910, respectively. The low level of Nei's genetic diversity and Shannon's Information Index using dominant AFLP markers in the domestic chicken breeds in general and WLH and RC in particular was due to the large proportion of monomorphic bands as 210 (78%) in WLH, 177 (63%) in AS and 224 (80%) in RC populations.

5.2.2. Population Specific alleles

A total of 29 population specific AFLP bands were observed in all 4 populations using 20 *Eco*RI/*Taq*I primer combinations. In RJF, 13 out of 20 Primer combinations generated 26 population specific bands and the number of population specific bands from these primer combinations was ranged from 1-5. In domestic

chicken breeds very low number of population bands could be detected. 2 bands in RC and one band in AS were observed. While in WLH population no band was observed as population specific. The 14 out of 29 bands were present in less then 10 % allele frequency, all these bands were found in RJF only. In RJF, E01+T03 –09 AFLP band was found more important from the RJF identification point of view, as this band was present with allele frequency 0.3292. Another important band was found in AS, E02+T03 –08 with allele frequency 0.4226.

Knorr *et al.,* (1999) reported 209 jungle fowl specific AFLP bands using 36 PCs, in a backcross family derived from a Red Jungle Fowl by White Leghorn mating with White Leghorn as the recurrent parent. De Marchi *et al.*, (2005) reported breed-specific AFLP markers in four indigenous chicken breeds from the Veneto region of Italy. They detected only a few AFLPs as breed-specific in all four breeds. Ermellinata, Pe´poi and Robusta showed two breed-specific markers, and only one specific band was found for Padovana.

Mekchay *et al.,* (2005) used AFLP to assess the genetic diversity and specific marker between Thai native chickens and fast-growing broilers. Two AFLP fragments were identified as type-strains specific markers. With **E**-ACT/**T**-CAT primer combination, they found a band (270 bp) that was specific for slow-growing chickens, and another band (250 bp) that was specific for fast-growing chickens. Gao *et al.,* (2007) reported the breed specific bands in 12 Chinese indigenous breeds. They observed the highest number of specific bands 9 in Jiuyan black (C1) and Dongxiang black (C2) pooled DNA, and the lowest number 1 band in Wenchang (C6), and Xingyi bantam chickens (C9).

Therefore, the number of population specific AFLP bands observed by Knorr *et al.,* (1999) in jungle fowl was much higher than reported by us. However, De Marchi *et al.*, (2005) and Gao *et al.,* (2007) reported comparatively lower number of population specific AFLP bands in their study as detected by us.

5.2.3. Genetic differentiation and Gene flow estimation

The G_{ST} index (Nei's 1987) was used to estimate the percentage of the total variation accounted for by the between-Population component. The G_{ST} values estimation between RJF and the domestic chicken populations namely as WLH, AS and RC was observed 0.2502, 0.2032 and 0.2723 respectively. These estimates suggest that the genetic variation explainable due to population differences was

lowest between RJF and AS (~20%), while between RJF and RC, this component was 27%. Similarly, lower proportion of total genetic variation explainable due to population differences between AS and RC (~21%) was observed, while this component was maximum between WLH and RC (31%). However, the G_{ST} value across loci of all 20 PCs and across all the populations was 0.3246, indicating that about 32% of the total genetic diversity was observed among populations, while the remaining 68% was accounted by the within population component of variation. The G_{ST} value evaluated in our study was more similar to the G_{ST} value (0.401) reported by De Marchi *et al.*, (2005) in their study on four indigenous chicken breeds from the Veneto region of Italy using amplified fragment length polymorphism (AFLP) markers.

The gene flow was calculated as per Nei's 1987 by using the G_{ST} values estimated between Population-pairs and across all populations. The gene flow estimates ranged from 1.11 between WLH and RC populations to 1.96 between RJF and AS populations. The gene flow estimation between RJF and domestic chicken populations namely as WLH, AS and RC was observed 1.4984, 1.9600, and 1.3362 respectively. When we compared the gene flow between RJF and domestic chicken breeds, AS showed maximum gene flow with RJF, while the RC showed least. Within domestic chicken groups, the gene flow was maximum between AS and RC (1.9069), while it was least between WLH and RC (1.1106). In our study, we observed lower value of gene flow between population-pairs due to large genetic differences or G_{ST} value between all population-pairs.

Across all the loci and populations, gene flow was 1.0405. Musa *et al.*, (2007) reported comparatively higher gene flow (1.1890) among RJF (*Gallus gallus Spadiceus*), Rugao, Anka, Wenchang and Silikes based on polymorphism analysis in functional apo VLDL-II gene by RFLP and SSCP markers. The higher gene flow might be attributed to comparatively lower degree of genetic differentiation between these populations as the estimates were based on RFLP and SSCP, which are comparatively less informative in comparison to AFLP.

5.2.4. Genetic distance and phylogenetic analysis

The pair wise GI and GD was estimated as per Nei's 1972. RJF showed comparatively lower genetic identity estimates with the domestic chicken breeds (0. 8017 to 0.8377) in comparison to the genetic identity estimates between domestic

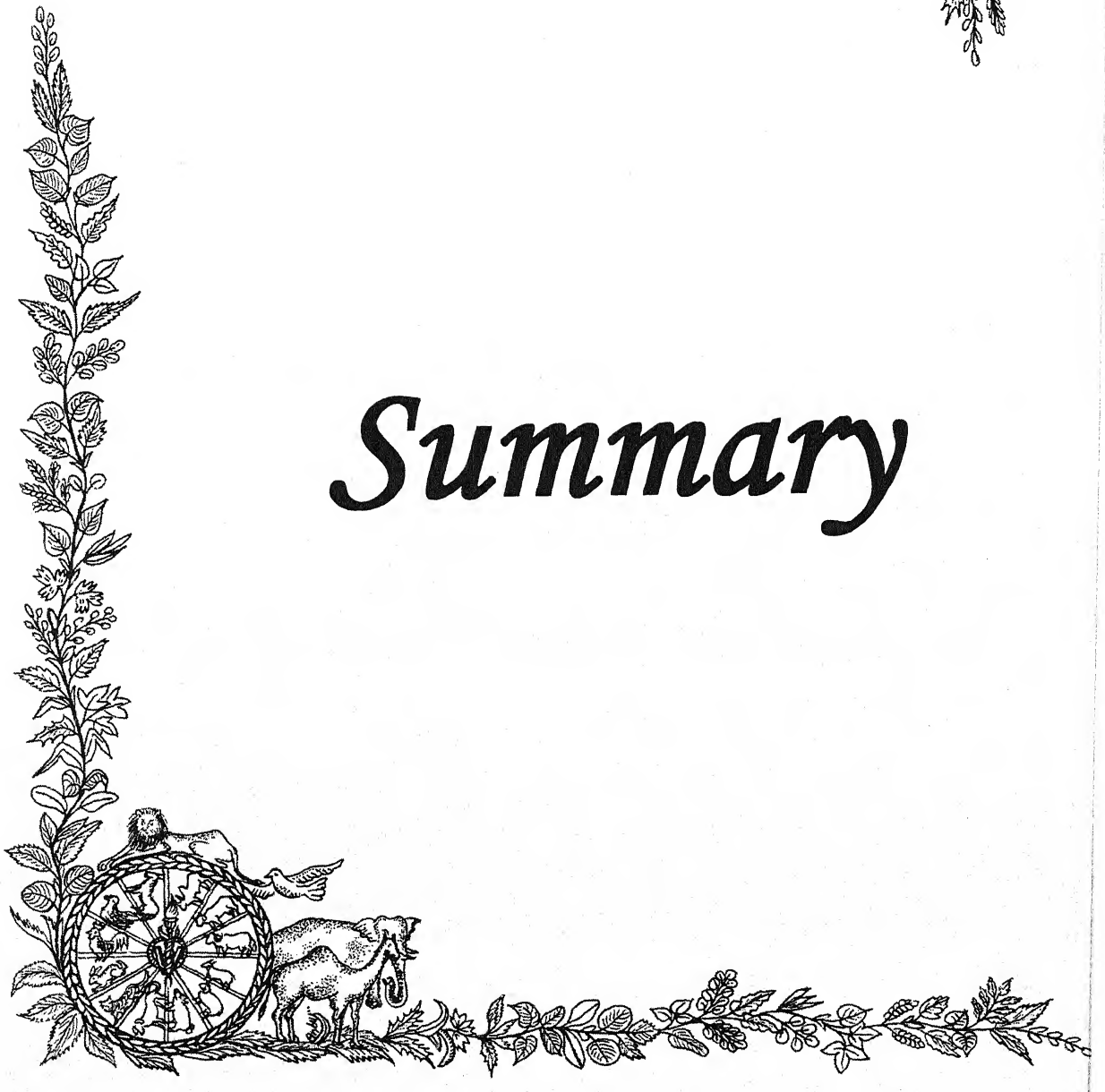
chicken breeds (0.9343 to 0.9442). Among the domestic chicken breeds, RJF showed least genetic identity with WLH, while maximum genetic identity with AS. The genetic distances between the RJF and domestic chicken breeds (0.1771 to 0.2210) were comparatively higher than those estimated among the chicken breeds (0.0575 to 0.0680) and among the chicken breeds, RJF showed least genetic distances with AS. Tomar *et al.,* (2007) also reported lower genetic distances between RJF and AS using three marker systems as RAPD, MASA and microsatellite.

In present study, much lower genetic distances (0.0575 to 0.0680) were reported between three chicken breeds i.e. WLH, AS and RC. While, Gao *et al.,* (2007) reported comparatively lower genetic similarity coefficients, ranging from 0.635 to 0.860 between 12 Chinese indigenous chicken breeds using six AFLP primer combinations. De Marchi *et al.*, (2005) reported genetic distances of 0.164 to 0.259 between four indigenous chicken breeds (Ermellinata, Padovana, Pe´poi and Robusta) from the Veneto region of Italy using amplified fragment length polymorphism (AFLP) markers. These higher genetic distances between chicken populations might possibly be due to the more diverse background of these populations.

A phylogenetic tree of RJF and three domestic chicken breeds was constructed based on Nei's standard genetic distances (1972) using UPGMA method. The RJF was quite distinct from domestic chicken breeds and formed a separate group whereas the three domestic chicken breeds were clustered together in a separate group. Within domestic chicken breeds, while WLH was placed in separate branch, RC and AS were placed together in one cluster. These results indicated that all domestic chicken breeds showed closed relationships with each other, while the RJF is almost equally distant from them. The RC was found more close to AS (game type). These results supported by Moiseyeva *et al.,* (2003) as they suggested that the meat type breeds to have descended from game breeds.

Another UPGMA based dendrogram among the 76 individuals of all four chicken populations revealed several interesting findings. While all the individuals of WLH and RC felt in separate, but one cluster, in AS, several clusters were observed. In RJF, nine out of 20 RJF individuals formed 7 separate clusters and the remained 11 individuals form a separate cluster as a sub group with domestic chicken breeds. This concluded that while the WLH and RC populations were more homogenous, AS population was more diverse and the RJF population is extremely diverse than the domestic chicken breeds. The high homogeneity in WLH and RC is very well

expected as both the populations are being maintained as a closed flock population since more than 30 generations and are under continuous selection for high performance. Comparatively, more diversity in AS population may be attributed due to the region that this population was though is being maintained as closed flock, but is not under any kind of selection. The extreme diversity in RJF population may clearly be attributed to the vast diverse background of this population.



Summary

Summary

In the present study, we studied the genetic polymorphism between red jungle fowl and domestic chicken, represented by three breeds i.e. White Leghorn (WLH) as egg-type, Aseel (AS) as game-type and Red Cornish (RC) as meat-type breeds by using two type of DNA markers i.e. microsatellite and AFLP markers and identified population specific private alleles for RJF as well as other chicken breeds. A resource population of 20 RJF, 20 WLH, 18 AS and 18 RC birds was made. The genomic DNA was isolated using phenol-chloroform extraction method and the DNA samples were diluted to the concentration of 25-50 ng/µl.

A total of 15 tetra-nucleotides and 10 di-nucleotides polymorphic microsatellite markers were used to genotype the resource population. The PCR products for 23 markers were resolved on 3.5% metaphor agarose, stained with ethidium bromide and viewed under UV light, while the PCR products from 2 markers i.e. MCW111 and LEI192 were resolved on Automated DNA Sequencer (3130xl Genetic Analyzer from Applied Biosystems).

All the 25 microsatellite loci were polymorphic within as well between the populations, except Locus ADL120 in WLH and RC, where only one allele i.e. 156 bp in WLH and 152 bp in RC was observed. A total of 475 alleles with an average of 19 alleles per locus were produced with 25 microsatellite markers. The highest number of 248 alleles with an average of 9.92 alleles per locus were observed in RJF followed by 212 alleles with an average of 8.48 alleles per locus in AS, 177 alleles with an average of 7.08 alleles per locus in each of WLH and RC populations. The number of alleles at each locus ranged from 5 (MCW111) to 43 (LEI212).

Expected heterozygosity (H_E) per locus ranged from 0.7285 (MCW111) and 0.9450 (LEI212), whereas across all loci, it was 0.8830. The range of observed heterozygosity (H_O) in this study was between 0.1486 (ADL120) and 0.6842 (LEI234), whereas across the all loci, it was 0.4344. Similarly, The PIC value per locus ranged from 0.6814 (MCW111) and 0.9426 (LEI212), whereas across all loci, it was 0.8715.

In RJF, the expected as well as observed heterozygosity and PIC pooled over all the loci were 0.7975, 0.4565 and 0.7716, respectively. Similarly in WLH, the expected as well as observed heterozygosity and PIC pooled over all the loci were 0.6946, 0.3822 and 0.6576, respectively. While in AS, the expected as well as observed heterozygosity and PIC pooled over all the loci were 0.7396, 0.4492 and 0.7112, respectively, in RC the respective figures were 0.6958, 0.4550 and 0.6584. Although varying among populations, mean observed heterozygosity was lower than the mean expected heterozygosity for all the populations.

A total of 242 population specific alleles with allelic frequency ranged from 0.0250 to 0.8333 were detected across the RJF and three domestic chicken breeds with all 25 microsatellite loci. Out of these 242 population specific alleles, 103 were specific to RJF, 44 were specific to WLH, 56 were specific to AS and 39 were specific to RC. Out of 242 population specific alleles, majority of the alleles (~75 %) were present in low to very low frequency and only 61 alleles were found in a frequency of $\geq$ 0.10. Among these 61 alleles, we found 29 population specific alleles in the range of allelic frequency 0.100 to 0.500 in RJF, 12 in RC (0.111 to 0.647), 10 in WLH (0.100 to 0.575) and also 10 in AS (0.100 to 0.833). Among the 29 RJF specific alleles, only 4 alleles, i.e. ADL120-178 bp, ADL265-114 bp, MCW111-104 bp and MCW111-106 bp seemed to have some practical importance as these alleles are present in very good allelic frequency range of 0.500 to 0.526. In domestic chicken breeds, three alleles i.e. LEI243-186 bp in WLH, LEI214-140 bp in AS and LEI229-193 bp in RC were more important from the breed identification point of view. Considering all the chicken population together, there are 226 alleles, which are present in domesticated chicken, but absent in RJF.

Genetic differentiation was examined by fixation indices F_{IS}, F_{IT} and F_{ST} for each locus across populations and among population pairs. In present study, the F_{IS} estimates were ranged from 0.1449 to 0.6615 at different microsatellite loci, however across all the loci, the F_{IS} was 0.4047. The F_{IT} estimates at each locus ranged from 0.2649 to 0.8024, while across all the loci, the estimate was 0.5068. The F_{ST} estimates, which also represent the fixation coefficient of sub populations ranged from 0.0580 (ADL 254) to 0.5530 (ADL 120) with a mean of 0.1716 across loci. This mutilocus F_{ST} value (0.1716) indicated that around 17% of total genetic variation was explained by differences among populations while the remaining 83% corresponding to differences among individuals within population.

Pair-wise F_{ST} among all population-pair of RJF and different domestic chicken populations ranged from 0.1027 to 0.1443. The F_{ST} estimates between RJF and domestic chicken breeds were lower (0.1027 to 1.1224) in comparison to those among the domestic chicken breeds (0.1278 to 0.1443). Among the domestic chicken breeds, RJF showed lowest F_{ST} with AS (0.1027) and highest with RC i.e. 0.1224.

Among Red Jungle Fowl and domestic chicken breeds, in present study, the Gene flow (Nm) varied from 0.2021 (ADL120) to 4.0612 (ADL254) with a mean of 1.2070 across all loci. Pair-wise gene flow among RJF and domestic chicken populations, the gene flow estimates ranged from 1.4822 to 2.1840. The gene flow estimates between RJF and domestic chicken breeds were higher (1.7921 to 2.1840) in comparison to those among the domestic chicken breeds (1.4822 to 1.7057). Among the domestic chicken breeds, RJF showed maximum gene flow estimates of 2.1840 with AS and lowest with RC i.e. 1.7921.

Genetic distances between the populations were estimated using shared allele frequencies. Genetic distance between RJF and domestic chicken populations namely as WLH, AS and RC was observed 0.7562, 0.7689 and 0.8093 respectively. Within domestic chicken populations genetic distances was observed 0.7637 (between WLH and RC) followed by 0.7495 (between WLH and AS) and 0.7302 (between AS and RC). The RJF showed maximum genetic distance with RC and minimum genetic distance with WLH. Among domestic populations, maximum genetic distance was observed between WLH and RC and minimum between AS and RC. The UPGMA dendrogram based on shared allele distances clearly clustered all 4 populations to understand relationships between RJF and domestic chicken breeds. Broadly, All four populations were divided in to two main clusters. The RJF and WLH were placed in cluster-I and AS and RC were placed in cluster-II.

In the AFLP analysis, we used two restriction enzymes a rare cutter (*Eco*RI) and a frequent cutter (*Taq*I). The pre-amplification was done by using *Eco*RI primer +1nt and *Taq*I primer +1nt (E+1 and T+1, while the selective amplification was done by using 4 *Eco*RI Selective amplification primers and 6 *Taq*I Selective amplification primers. In total 20 primer combinations were used. A total of 318 scorable AFLP bands in the range of 50-500 bp were detected across the populations with 20 *Eco*RI/*Taq*I primer combinations (PCs). Out of which, 309 (97.17%) were polymorphic bands. The mean number of polymorphic bands across all 4 populations was 15.4 per Primer Combination (PC). The extant of polymorphism varied within the

populations as well as with each primer combinations. In RJF population, a total of 311 bands could be scored. Out of which, 302 (97%) were found polymorphic. Among the primer combinations the extant of polymorphism was 85% to 100%. In WLH population, a total of 270 bands could be scored. Out of which, 60 (22%) were found polymorphic. In AS population, a total of 282 bands could be scored. Out of which, 105 (37%) were found polymorphic. In RC population, a total of 281 bands could be scored. Out of which, 57 (20%) were found polymorphic. The mean polymorphic bands across all 20 primer combination were 15.1 in RJF, 3.0 in WLH, 5.2 in AS and 2.8 in RC

A total of 29 population specific AFLP bands were observed in all 4 populations using 20 *Eco*RI/*Taq*I primer combinations. In RJF, 13 out of 20 Primer combinations generated 26 population specific bands and the number of population specific bands from these primer combinations was ranged from 1-5. In domestic chicken breeds very low number of population bands could be detected. 2 bands in RC and one band in AS were observed. While in WLH population no band was observed as population specific. The 14 out of 29 bands were present in less then 10 % allele frequency, all these bands were found in RJF only. In RJF, E01+T03 –09 AFLP band was found more important from the RJF identification point of view, as this band was present with allele frequency 0.3292. Another important band was found in AS, E02+T03 –08 with allele frequency 0.4226.

Mean Nei's genetic diversity (h) across all loci generated by all 20 PCs was maximum in RJF (0.3850) followed by in AS (0.1287). In WLH and RC, the h estimates were quite low (0.0669 and 0.0656, respectively). Similarly the means of Shannon's Information Index (I) across all loci generated by all 20 PCs was maximum in RJF (0.5529), followed by AS (0.1889) and were quite low in WLH and RC (0.0993 and 0.0968, respectively).

The G_{ST} index (Nei's 1987) was used to estimate the percentage of the total variation accounted for by the between-Population component. The G_{ST} values estimation between RJF and the domestic chicken populations namely as WLH, AS and RC was observed 0.2502, 0.2032 and 0.2723 respectively. These estimates suggest that the genetic variation explainable due to population differences was lowest between RJF and AS (~20%), while between RJF and RC, this component was 27%. Similarly, lower proportion of total genetic variation explainable due to population differences between AS and RC (~21%) was observed, while this

component was maximum between WLH and RC (31 %). However, the G_{ST} value across loci of all 20 PCs and across all the populations was 0.3246, indicating that about 32% of the total genetic diversity was observed among populations, while the remaining 68% was accounted by the within population component of variation.

The gene flow was calculated as per Nei's 1987 by using the G_{ST} values estimated between Population-pairs and across all populations. The gene flow estimates ranged from 1.11 between WLH and RC populations to 1.96 between RJF and AS populations. The gene flow estimation between RJF and domestic chicken populations namely as WLH, AS and RC was observed 1.4984, 1.9600, and 1.3362 respectively. Within domestic chicken groups, the gene flow was maximum between AS and RC (1.9069), while it was least between WLH and RC (1.1106). Across all the loci and populations, gene flow was 1.0405.

The pair wise GI and GD was estimated as per Nei's 1972. RJF showed comparatively lower genetic identity estimates with the domestic chicken breeds (0. 8017 to 0.8377) in comparison to the genetic identity estimates between domestic chicken breeds (0.9343 to 0.9442). Among the domestic chicken breeds, RJF showed least genetic identity with WLH, while maximum genetic identity with AS. The genetic distances between the RJF and domestic chicken breeds (0.1771 to 0.2210) were comparatively higher than those estimated among the chicken breeds (0.0575 to 0.0680) and among the chicken breeds, RJF showed least genetic distances with AS.

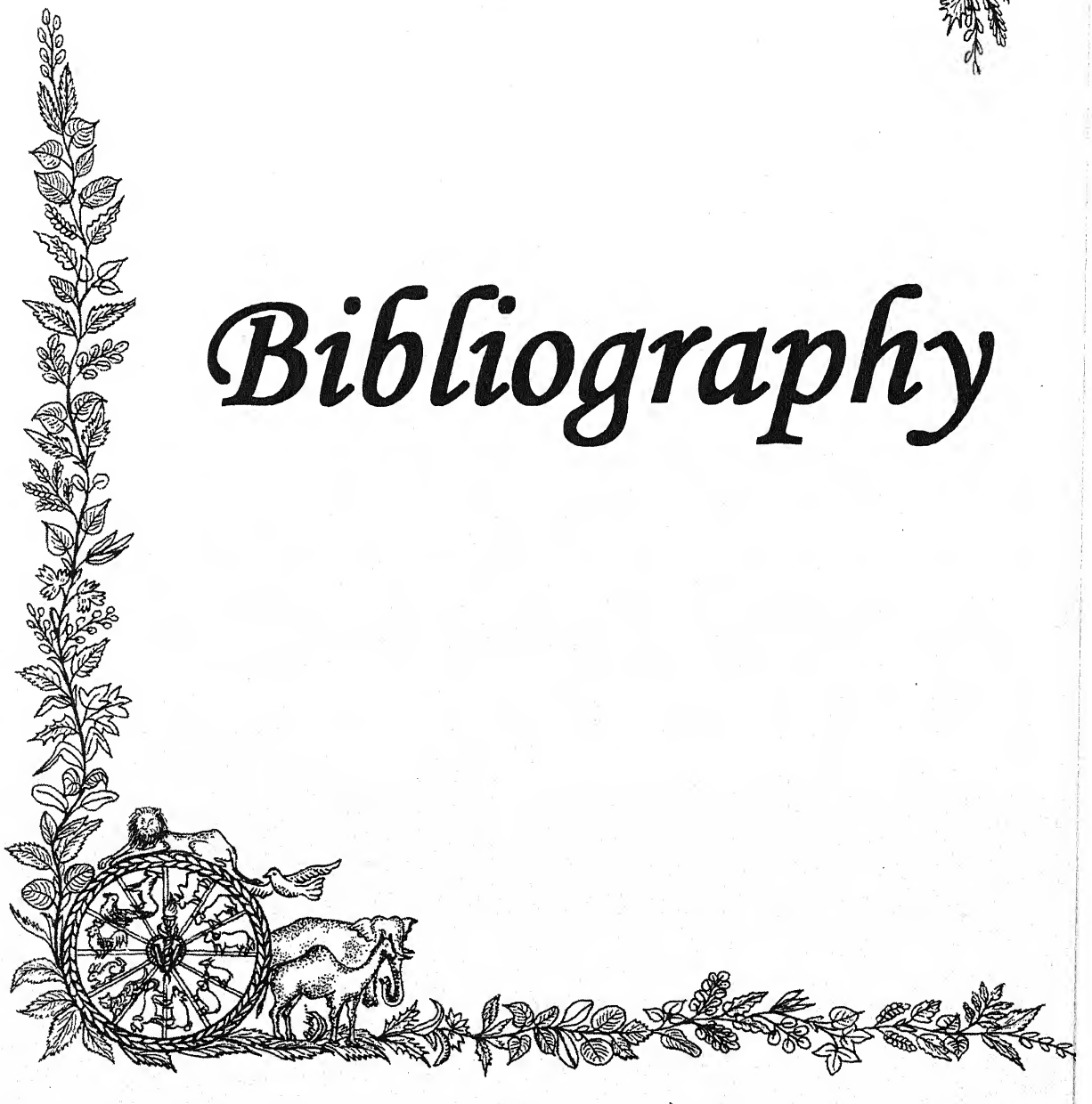
A phylogenetic tree of RJF and three domestic chicken breeds was constructed based on Nei's standard genetic distances (1972) using UPGMA method. The RJF was quite distinct from domestic chicken breeds and formed a separate group whereas the three domestic chicken breeds were clustered together in a separate group. Within domestic chicken breeds, while WLH was placed in separate branch, RC and AS were placed together in one cluster. Another UPGMA based dendrogram among the 76 individuals of all four chicken populations revealed several interesting findings. While all the individuals of WLH and RC felt in separate, but one cluster, in AS, several clusters were observed. In RJF, nine out of 20 RJF individuals formed 7 separate clusters and the remained 11 individuals form a separate cluster as a sub group with domestic chicken breeds.

Hence, the following inferences may be drawn from present study-

- All the 25 microsatellite markers identified for genotyping were polymorphic between the populations.

- A total of 475 alleles were amplified with 25 microsatellite markers and all were polymorphic between populations.
- In RJF maximum alleles (248 alleles) were amplified while in exotic breeds i.e. WLH and RC, lowest number of alleles (177 alleles) were amplified.
- Expected heterozygosity of 0.8830 across all loci suggested the suitability of these markers in detecting polymorphism between the populations.
- The high PIC values at the majority of microsatellite loci also suggested the effectiveness of the markers used in present study.
- The observed heterozygosity across all the loci ranged from 0.3822 (in WLH) to 0.4565 (in RJF) and was lower than the expected heterozygosity suggesting deficit of the heterozygotes at these loci.
- Out of the 475 alleles amplified, 242 were found to be population specific alleles, however majority of them (~75 %) had very low frequency (<10 %).
- Out of the 61 population specific alleles present with a frequency of ≥ 10 %, only 4 alleles, i.e. ADL120-178bp, ADL265-114bp, MCW111-104 bp and MCW111-106 bp in RJF, three alleles i.e. LEI243-186 bp in WLH, LEI214-140 bp in AS and LEI229-193 bp in RC seemed to have some practical utility.
- The gene flow estimates between RJF and domestic chicken breeds were higher (1.7921 to 2.1840) in comparison to those among the domestic chicken breeds (1.4822 to 1.7057). Among the domestic chicken breeds, RJF showed maximum gene flow estimates of 2.1840 with AS and lowest with RC i.e. 1.7921.
- The dendrogram based on shared microsatellite allele distances showed the clustering of RJF and WLH in one cluster, while AS and RC was placed in second cluster.
- AFLP analysis was done by using *Eco*RI and *Taq*I. In total 20 primer combinations were used.
- Out of the 318 scorable AFLP bands, 309 (97.17%) were polymorphic bands.
- In RJF population, 302 out of the total 311 bands scored were polymorphic.
- In domestic chicken breeds, 20% to 37 % bands were polymorphic.
- A total of 29 population specific AFLP bands were observed using 20 *Eco*RI/*Taq*I primer combinations. Out of these, 26 were in RJF.

- The G_{ST} index value across loci was 0.3246, while the gene flow across all the loci and populations was 1.0405.
- A phylogenetic tree based on Nei's standard genetic distances (1972) showed that RJF formed a separate group whereas the domestic chicken breeds were clustered together in a separate group.



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Appendix-I

2 M Tris-HCl

Tris	242.2 g

Dissolve in 800 ml of autoclaved Distilled water (DW) adjust the pH 8.0 by HCl. Make volume to 1000 ml. Sterilize by autoclave and store at 4°C.

0.5 M EDTA

EDTA	186.1 g

Add 186.1 g of disodium EDTA.$2H_2O$ to 800 ml of autoclaved DW. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (approx. 20 g of NaOH pellets) and make up volume to 1000 ml with DW. Dispense into aliquots and sterilize by autoclaving and store at 4°C.

Note: The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approx. 8.0 by the addition of NaOH.

5 M NaCl

NaCl	292.9 g

Dissolve in 800 ml of distilled water on magnetic stirrer and make volume up to 1000 ml. Autoclave and store at room temperature.

10% Sodium Dodecyl Sulphate

SDS	10. g
Autoclaved DW	100 ml

Store at room temperature, Heat before use at 60°C.

3 M Sodium Acetate

Sodium Acetate	408.3 g

Dissolve 408.3 g of sodium acetate.$3H_2O$ in 800 ml of H_2O. Adjust the pH to 5.2 with glacial acetic acid or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving and store at room temperature.

Lysis Buffer (pH 8.0)

2 M Tris HCl (pH 8.0)	0.5 ml
0.5 M EDTA (pH 8.0)	0.2 ml
2 M NaCl	2.0 ml
10 % SDS	5.0 ml

Add autoclaved DW up to 100 ml and store at 4°C.

Tris Saturated Phenol

1. Melt phenol at 68°C by keeping on water bath.
2. Measure the required amount. Add 8-hydroxyquinoline at a final concentration of 0.1% (It is an antioxidant, gives yellow color to phenol).
3. Extract phenol several times with equal volume of 1 M Tris (pH 8.0).
4. Then with 0.1 M Tris, until the pH of the aqueous phase is more than 7.6.
5. Add 0.2 % β-mercaptoethanol.
6. Mix thoroughly and store in amber colored bottle at 4°C.

Chloroform-Isoamyl alcohol. (24:1)

Chloroform	24 ml
Isoamyl alcohol	01 ml

Mix thoroughly and store at 4°C.

20 mg/ml Proteinase-K

Proteinase K	20 mg

Dissolve in 1 ml autoclaved D. W.

DW Store at –20 °C

Phenol- Chloroform-Isoamyl alcohol

Tris saturated phenol	25 ml
Chloroform- Isoamyl alcohol	25 ml

Mix thoroughly and store at 4 ^{0}C

Tris-EDTA Buffer (T:E :: 10:1)

2M Tris-HCl	250 ml
0.5 M EDTA	100 µl

Volume makes up with autoclaved distilled water up to 500 ml and store at 4°C.

20 X Tris Borate EDTA (TBE):

Tris base	216.0 g
Boric acid	110.0 g
0.5 M EDTA (pH 8.0)	80.0 ml
Autoclaved distilled water	1000 ml

Sterilize by autoclave and store at room temperature. The 0.5X working solution is 45 mM Tris-borate/1 mM EDTA.

50 X Tris Acetate EDTA (TAE):

Tris base	242.0 g
Glacial acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100 ml

Autoclaved distilled water up to 1000 ml.

Autoclave and store at room temperature. The 1X working solution is 40 mM Tris-acetate/1 mM EDTA.

Ethidium Bromide (10mg/ml):

Ethidium bromide	10 mg
D. W.	1.0 ml

Store it in dark by wrapping the tube with aluminum foil.

6X Loading Buffer:

Bromophenol blue	0.25% (w/v)
Sucrose	40.00% (w/v)

Store at 4°C.

Appendix - II

Protocol for AFLP reaction set up in poultry

1. Template Preparation

1.1. Restriction Digestion (RD) of DNA

1.1.1. Incubate 400 ng of genomic DNA (10 µl) + 15 µl of the *Taq*I RD mix **(Table1.1)** for 1 h at 65 °C and mix gently.

Table-1.1. *Taq*I RD Mix

*Volume to be add (µl) for number of samples (X)

Components	**X=1**
*Taq*I (20U/ µl)	0.25
10X RL buffer	2.50
Water	12.25
Final Volume	**15 µl**

1.1.2. Now 25 µl of solution **(step- 1.1.1)** + 15 µl *Eco*RI RD mix **(Table-1.2)** total 40 µl incubate for 1 h at 37 °C

Table-1.2. *Eco*RI RD mix

*Volume to be add (µl) for n.o. of samples (X)

Components	**X=1**
*Eco*RI (10U/ µl)	0.50
10X RL buffer	1.5
Water	13.0
Final Volume	**15 µl**

Reaction Ligation buffer:- 8.0 µl 5X-Pharmacia "One-Phor-All+" buffer [10X = 100 mM Tris-acetate, pH-7.5, 100 mM Mg-acetate, 500 mM K-acetate)

! CAUTION Prolonged incubation with the restriction enzyme *Eco*RI (e.g., overnight) is not recommended because of its possible **'star'** activity, giving reduced cleavage specificity and, ultimately, aberrant AFLP fingerprints.

1.2. Adapter Preparation

1.2.1. *Eco*RI-adapter-

mix 10 pmol *Eco*RI- adaptor top strand (volume- 10 µl) with 10 pmol *Eco*RI- adaptor bottom strand (volume- 10 µl) plus 1 µl 10X Tango buffer, to make 21µl final volume, heat to 95°C, and allow to cool to room temperature (RT) slowly. This gives a final concentration (near about) of 5 pmol/µl and makes enough adapters for 21 ligations.

1.2.2. *Taq*I-adapter

- mix 10 µl of 100 pmol *Taq*I- adaptor top strand and 10 µl of 100 pmol *Taq*I- adaptor bottom strand, 1 µl 10X Tango buffer to make 21µl final volume, heat to 95°C, and allow to cool to RT slowly. This gives a final concentration (near about) of 50 pmol/µl and makes enough adapters for 21 ligations.

[Note: Adapter strands should not be phosphorylated, this prevents adapter self ligation. Both adapters are engineered such that the ligation "kills" the restriction site to which the adapter is ligated.]

1.3. Adapter Ligation

Add **10 µl ligation mix** as detailed in **Table 1.3** and continue the incubation for another **3 h at 37 °C.**

Note: Do not inactive the restriction enzymes before the ligation.

Table-1.3. Ligation Mix

Components	**X=1**
*Eco*RI adapter (5 pmol/ µl)	1
*Taq*I adapter (50 pmol/ µl)	1
10X RL buffer	1
T4 DNA Ligase(5U/ µl)	0.2
ATP (10mM)	0.5
Water	6.3
Final Volume	**10 µl**

Total reaction volume = 50 µl

1.4. After ligation, dilute the reaction mixture to 200 µl with TE (10:1) buffer. This will serve now as template for the pre-amplification reaction.

- **If necessary, the template can be stored for up to 1 year at 20 °C.**

2. Pre-amplification of template DNA

2.1. Add 45 µl of pre-amplification mix **(from table 2.1)** to 5µl of the AFLP template (from step-1) and place in thermal cycler for PCR as reaction profile in **table-2.2.**

Table-2.1. Pre-amplification Mix

Components	X=1
10X PCR buffer	5.0
$MgCl_2$(25mM)	5.0
dNTPs mix (2.5mM)	4.0
*Eco*RI primer (E+1) 10 pmol/µl	1.5
*Taq*I primer Pre(50ng/ µl) or 10 pmol/µl	1.5
Ampli *Taq* (5U/µl)	0.2
Water	27.8
Final Volume	**45**

Table-2.2. "Pre-Amplification" thermocycle profile

Cycle number	Denature	Anneal	Extend	Final extend (optional)
1-25	94°C- 30 s	56°C -1 min	72°C – 1 min	5 minutes

2.2. Testing of Pre-amplified Product:- Run 5 µl of the pre-amplification reaction product on a 1% agarose gel in 1X TAE running buffer at 100 V for 10–15 min. Use 100 bp ladder as molecular weight .Use EtBr, DNA stain to visualize the pre-amplification products. Substantial smearing in the range of 50–500 bp indicates a successful pre-amplification PCR.

2.3. Dilute the pre-amplification reaction product 20-fold with T:E (10:1) buffer. These diluted reaction products serve as **templates for the final selective amplification reactions** using primers with two or three/four selective bases in one or both primers.

- **It can be stored for up to 1 year at 20 °C.**

3. Selective Amplification

3.1. 15 µl of selective amplification mix **(from table-3.1)** + 5 µl pre-amplification products from **step 2. and** Placed this reaction in thermal cycler for PCR as reaction profile in **table-3.2.**

Table- 3.1. Selective -amplification Mix

Components	**X=1**
10X PCR buffer	2.00
$MgCl_2$ (25mM)	2.00
dNTPs mix (2.5mM)	1.60
EcoRI labeled primer 2 pmol/µl	0.50
TaqI primer slective 10 pmol/µl	0.60
Ampli Taq (5U/µL)	0.12
Water	8.18
Final Volume	**15**

Table- 3.2. PCR Profile for selective amplification

Cycle number	**Denature**	**Anneal**	**Extend**
1-2	94°C- 30 s	66°C -30 s	72°C –1 min
3-4 (next two)	94°C- 30 s	64°C -30 s	72°C –1 min
5-6 (next two)	94°C- 30 s	62°C -30 s	72°C –1 min
6-8 (next two)	94°C- 30 s	60°C -30 s	72°C –1 min
9-11 (next two)	94°C- 30 s	58°C -30 s	72°C –1 min
12-36 (next 25)	94°C- 30 s	56°C -30 s	72°C –1 min
37			72°C –5 min

OR

Cycle number	**Denature**	**Anneal**	**Extend**
1-13	94°C- 10 s	65°C -30 s each cycle reduce by 0.7°C	72°C –1 min
14-36	94°C- 30 s	56°C -30 s	72°C –1 min
37			72°C –2 min

References : Vos *et al.,* (1995); Vuylsteke *et al.,* (2007)